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GRANT NUMBER DAMD17-95-1-5064

TITLE: Study of Chlamydia trachomatis in Military Women; Prevalence, Risk Factors, and a Cost Benefit Analysis of Early Diagnosis and Treatment

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REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 1998	3. REPORT TYPE AND DATES COVERED Annual (31 Aug 97 - 30 Aug 98)	
4. TITLE AND SUBTITLE Study of Chlamydia trachomatis in Military Women; Prevalence, Risk Factors, and a Cost Benefit Analysis of Early Diagnosis and Treatment			5. FUNDING NUMBERS DAMD17-95-1-5064	
6. AUTHOR(S) Charlotte A. Gaydos, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Johns Hopkins University Baltimore, Maryland 21205-2196			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, MD 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) In the third project year, 5,657 women and 1,203 male military recruits were screened at Fort Jackson, SC. Over the course of the project, there have been 16,727 women and 1,203 men screened. Those that tested positive received treatment at the Troop Medical Clinic (TMC). Methods: After receiving an hour of instruction on chlamydia including symptoms and sequelae, prevention, and screening and treatment, potential subjects were asked for their informed consent. All potential subjects answered a chlamydia risk history questionnaire, however, some who chose each month not to participate submitted the questionnaire anonymously. Study participants submitted a first catch urine specimen for testing at Johns Hopkins by ligase chain reaction (LCR). Results: 583 women (10.3%) and 59 men (4.9%) tested positive and were subsequently treated. If a questionnaire could be avoided and young age (≤ 25 years) used alone as the screening criterion, 87.9% (11,603/13,204) of the population would need to be tested, and 95.3 % (1,162/1,219) of the positives would be identified. Young age was not a risk factor for men, as it was for women. Conclusion: We recommend screening women ≤ 25 years of age in this population.				
14. SUBJECT TERMS Defense Women's Health Research Program			15. NUMBER OF PAGES 70	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

FOREWORD

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Charlitta Gaydos, DrPH. 7-27-98
PI - Signature Date

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5 Introduction

Each year in the United States, there are approximately 4.5 million cases of *Chlamydia trachomatis* infection¹. Although many *C. trachomatis* infections are asymptomatic² the symptoms and sequelae from infection, including cervicitis, urethritis, chronic pelvic pain, pelvic inflammatory disease (PID), ectopic pregnancy, and infertility represent a large disease burden^{1,3}. Infection rates for young, sexually active women range from 5-20%. Infants infected perinatally can develop pneumonia, otitis media, and conjunctivitis (if not given ocular prophylaxis)^{1,4,5}. In men, chlamydia infections can cause urethritis, epididymitis, and proctitis^{1,4,5}.

C. trachomatis can be transmitted through vaginal, anal, and oral sex as well as mother-to-infant. Risk factors in women for infection include having cervical ectopy, being a young female, having multiple sex partners, inconsistent condom use^{1,6,7,8}. In men risk factors include having multiple sex partners, engaging in unprotected sex, and having multiple sex partners¹.

Detection of genital *C. trachomatis* infection by ligase chain reaction (LCR) using first void urine is a non-invasive, highly sensitive, and highly specific procedure^{9,10}. Although the cost of LCR is higher than other tests such as direct fluorescent antibody (DFA), antigen detection by enzyme immunoassay (EIA), and nucleic acid probe tests, it is more sensitive and more specific^{11,12}. Culture has been considered to be the gold standard in the past, but costs more and is less sensitive than either LCR or PCR^{9,13-17}.

In the U.S., the annual costs of chlamydia infections and their sequelae are estimated at \$5 billion. Screening with the urine LCR for the detection of *C. trachomatis* and treatment of those persons testing positive with a single dose therapy of azithromycin could prevent a large burden of disease caused by *C. trachomatis* infection.

This grant has been in progress for three years. Based upon findings of the study, recommendations will be made for an effective, cost-effective chlamydia control program designed to reduce morbidity due to *C. trachomatis*.

The objectives of this study are to;

1. Determine the prevalence of infection in several military populations at risk of *C. trachomatis* infection including: female military recruits, and women attending the Troup Medical Clinic (TMC) with urogenital symptoms or the Pap Smear Clinic,
2. Determine risk factors predictive of infection;
3. Conduct a cost-effectiveness analysis comparing universal screening versus selective screening utilizing risk factor criteria;
4. Recommend a chlamydial control program: selective screening and treatment, universal screening and treatment, or mass therapy for all female basic recruits; and
5. Monitor PID and ectopic pregnancy rates, over the 3 year period of chlamydia screening.

Determination of the prevalence of chlamydia in the TMC and Pap Clinics were completed in previous study years. Data regarding these portions of the grant were analyzed for and reported in previous annual reports.

This year, a number of study objectives were achieved. Incoming female recruits were screened and the data were analyzed for a third year. Finally, cost-effectiveness models were finalized to determine how best to prevent the sequelae from *C. trachomatis* infections in the upcoming projects' fourth year.

In addition to the aforementioned study objectives, we screened over one thousand incoming male recruits to estimate prevalence in men. Risk factors for infection in male recruits were also studied. This project was undertaken to determine, in part, reinfection potential of women undergoing the screening program.

From the previous three complete years of research, we make the recommendation that young age (≤ 25 years) would be a valid risk factor upon which to screen female recruits joining the U.S. Army.

6 Body

6.1 Methods

STUDY SUBJECTS

Every Sunday, recruits undergoing in processing at the Reception Battalion, Ft. Jackson were directed to a classroom to be taught about the transmission, symptoms, and sequelae of chlamydia by a research nurse. Potential subjects were then asked to participate in the study by filling out a demographic and risk behavior questionnaire and submit a urine specimen. Only female recruits were screened with the exception of the period from May 24, 1998 to June 14, 1998 when only male (n=1,203) recruits from the Physical Exam Section (PES) were screened.

After giving their informed consent, each subject completed a questionnaire for demographic information and sexual risk factor history and provided a urine sample. The questionnaire was a one page, two-sided scanning form (Scantron Corporation, Tustin, CA). All urine specimens, consent forms, and questionnaires were shipped to Johns Hopkins University Chlamydia Laboratory, under appropriate environmental conditions (4° C for urine specimens).

To determine comparability of the volunteer and non-volunteer recruits at the Reception Battalion, with regard to demographics and risk history, a sub-sample of those non-volunteering recruits were invited to anonymously fill out a questionnaire. This sub-sample was collected on the first Sunday of each month from female recruits and during all four weeks of male recruit screening.

CHLAMYDIA URINE LCR TESTS.

Urines were processed and tested by ligase chain reaction (LCR) (Abbott Laboratories, Abbott Park, IL) for chlamydial DNA, according to manufacture's directions. The LCR test is cleared by the FDA for use with female and male urine specimens.

DATA MANAGEMENT AND ANALYSIS

The scan forms were scanned into an Ascii text format and then appended to the main data base stored as an Access97 (Microsoft Corporation, Seattle, WA) file. The LCR results, demographics, and risk factor information were analyzed using chi-squared tests, Fisher's exact tests

and logistic regression analysis (Stata 4.0, College Station, TX). Data for the multivariate models were recorded as dichotomous variables (presence of risk vs. no risk or unknown risk) according to the findings of the univariate analysis.

6.2 Results

FEMALE RECRUITS, FT JACKSON, 1997-8 (3rd Year): Of 6,812 recruits presenting at the Physical Exam Station, 5,657 (83.0%) volunteered from August 31, 1997 to August 30, 1998. From the 5,657 participating female recruits, 88.5% (5007/5655) were age 25 or younger, 49.2% (2774/5640) were Caucasian, 35.1% (1979/5640) were African American, and 15.7% (887/5640) were other races. The prevalence for *C. trachomatis* by urine LCR for the population was 10.3% (583/5657).

FEMALE RECRUITS, FT JACKSON, 1996-7 (2 project years): A thorough analysis of this population from January 1996 to December 1997 was completed on 13,223 female subjects presenting at the Physical Examination Section (See Appendix 9.1.3 for the full article)¹⁸. The infection status of nineteen individuals could not be determined due to missing data items or insufficient urine. Of the remaining 13,204 female recruits, the median age was 21 years (range 17 to 39) and 87.9% (11,603/13,204) were age 25 or younger. Half of the women were Caucasian, 35.9% were African American, and 13.1% were other races. The prevalence for *C. trachomatis* by urine ligase chain reaction for the entire population was 9.2%.

By questionnaire, 93.1% reported having had vaginal sex, 26.7% had more than 1 sex partner in the previous 90 days, 31.4% had a new sex partner in the previous 90 days, and only 16.9% always used condoms. A prior history of chlamydia infection was reported in 9.1%, gonorrhea in 3.3%, syphilis in 0.6%, and trichomonas infection in 4.6%. Of those volunteers who denied vaginal sex, 1.4% (13/914) were chlamydia positive, and of those who reported always using condoms, 8.4% (177/2,115) were chlamydia positive.

Of the 823 non-volunteer recruits who filled out a questionnaire anonymously, 203 (24.7%) did not provide age data and were dropped from the analysis. Their mean age was 21 (range 17 to 36), 51.3% were Caucasian and 31.9% were African American and not significantly different from

volunteers. Only 66.9% reported having vaginal sex compared to 93.1% of the volunteers ($p < 0.001$). In the non-volunteers, four variables were significantly different from those of the volunteers, even when vaginal sex was controlled for: only 4.0% reported prior chlamydia infections ($p = 0.013$), 18.2% had a new sex partner ($p = 0.002$), 20.1% consistently used condoms ($p < 0.001$), and 90.7% reported no previous STD diagnosis ($p = 0.001$). Of the non-volunteers, 17.7% had more than one sex partner in the prior 90 days, similar to the volunteers when controlling for vaginal sex ($p = 0.189$).

The age-specific prevalence of *C. trachomatis* infection for the 13,204 volunteers is shown in Fig. 1¹⁸. The highest prevalence of chlamydia was in females aged 17 (12.2%) years. Prevalences declined sharply with increasing age to below 5% for ages older than 25 years. For further analysis, the youngest age categories (17 to 25 yr.) were combined into a variable called “young” (prevalence 10.0%, 1162/11,603). In the “older” category (ages 26 to 39 yr.) the prevalence was 3.6% (57/1,601). By race, prevalences were 5.5% (369/6,715) for Caucasian, 14.9% (707/4,733) for African-American, and 8.1% (143/1,756) for other races.

Univariate Analysis.

Univariate analysis identified 10 significant variables associated with chlamydia infection: young age (17 to 25 years), African-American race, ethnicity other than Caucasian or African American, ever having vaginal sex, > 1 sex partner in the previous 90 days, new sex partner in the previous 90 days, having inconsistently used condoms last 90 days, a previous diagnosis of gonorrhea, a previous diagnosis of trichomonas, and history of any sexually transmitted disease (Table 2)¹⁸. Prior diagnosis of chlamydia or syphilis were not significantly associated with being chlamydia positive.

Multivariate Analysis.

In the complete multivariate model, vaginal sex (odds ratio 5.9, 95% confidence interval 3.2, 10.6), young age (odds ratio 3.0, 95% confidence interval 2.3, 4.0), African American race (odds ratio 3.4, 95% confidence interval 2.9, 3.8), more than 1 sex partner (odds ratio 1.4, 95% confidence interval 1.2, 1.7), having a new sex partner (odds ratio 1.3, 95% confidence interval 1.1, 1.6), having inconsistently used condoms (odds ratio 1.4, 95% confidence interval 1.1, 1.6), and history of any

sexually transmitted diseases (odds ratio 1.2, 95% confidence interval 1.0, 1.4) were independent predictors for chlamydia infection (Table 3).

Strategies for Selective Screening Criteria for Identification of Chlamydia Infections.

A screening strategy using all variables identified as independent predictors would require testing 100% of the population, and would detect 100% of the positive individuals. In this model, the magnitude of risk associated with having a new sex partner may vary across race categories. For the purpose of a screening program, this would not alter the proportion of the population tested or the percent of positives detected using this model. Because screening based on race would likely be viewed as inequitable, a strategy excluding race was examined. This strategy would necessitate a questionnaire, and include those reporting high risk behaviors, such as more than one or a new sex partner in the previous 90 days, lack of condom use, prior sexually transmitted disease, or young age. Screening on these criteria would still require testing 100% of the population. If a questionnaire could be avoided and young age (≤ 25 years) used alone as the screening criterion, 87.9% (11,603/13,204) of the population would need to be tested, and 95.3 % (1,162/1,219) of the positives would be identified. We therefore recommend screening women ≤ 25 years of age only for the last year of the study.

MALE RECRUITS, FT JACKSON (1998): There were 1,203 men screened, the prevalence of infection was 4.9%. The mean age was 19.8 (± 2.61) years, 61.6% were Caucasian, 33.8% had more than one sex partner, and 36.7% had a new sex partner in the last 90 days. Only 21.2% of men reported using condoms regularly, and 2.6% reported having prior chlamydial infections. Of the men infected with *C. trachomatis*, only 13.6% reported having symptoms. Risk factors that proved useful for predicting chlamydial positivity included: African-American race, more than one sex partner, and a new sex partner. Young age was not a risk factor, as it was for women. This information was submitted to two conferences in abstract form (See Appendices 9.3.2 and 9.3.3).

CHLAMYDIA PREVALENCE BY PUBLIC HEALTH REGION ORIGIN OF RECRUITS:
The following table, Table 1, describes the geographic variability by the public health region origin

of the recruits for 1) females screened this third project year, 2) males screened this third project year, and 3) females from years one to three of the project. Figures in Appendices 9.4.1, 9.4.2, and 9.4.3.

Table 1. Chlamydia prevalence by public health region in study recruits; 1) female 3rd year, 2) females for 3 project years, and 3) males 3rd year.

Public Health Region	States within the Region	Chlamydia Prevalence (%) Females (3rd year)	Chlamydia Prevalence (%) Females (Total)	Chlamydia Prevalence (%) Males (3rd Year)
Region 1	Massachusetts, Connecticut, Rhode Island, Vermont, New Hampshire, Maine	7/154 (4.5)	19/486 (3.9)	1/20 (5.0)
Region 2	New York, New Jersey, Puerto Rico, Virgin Islands	61/613 (10.0)	153/1713 (8.9)	3/70 (4.3)
Region 3	Delaware, Maryland, Pennsylvania, Virginia, West Virginia, District of Columbia	62/664 (9.3)	150/1926 (7.8)	4/124 (3.2)
Region 4	Georgia, North Carolina, South Carolina, Florida, Alabama, Mississippi, Tennessee, Kentucky	214/1391 (15.4)	564/4328 (13.0)	22/340 (6.5)
Region 5	Illinois, Minnesota, Michigan, Indiana, Ohio, Wisconsin	54/667 (8.1)	153/2153 (7.1)	4/135 (3.0)
Region 6	Arkansas, Louisiana, New Mexico, Oklahoma, Texas	92/798 (11.5)	282/2350 (12.0)	12/166 (7.2)
Region 7	Nebraska, Missouri, Iowa, Kansas	15/251 (6.0)	44/684 (6.4)	3/83 (3.6)
Region 8	Colorado, Montana, North Dakota, South Dakota, Utah, Wyoming	7/138 (5.1)	17/469 (3.6)	1/31 (3.2)
Region 9	Arizona, California, Hawaii, Nevada, American Samoa, Guam, Mariana Islands, Marshall Islands, Micronesia, Palau	47/627 (7.5)	130/1674 (7.8)	4/92 (4.3)
Region 10	Oregon, Washington, Idaho, Alaska	11/221 (5.0)	24/647 (3.7)	1/22 (4.5)

DISCUSSION IN RELATION TO STATEMENT OF WORK AND PROBLEMS.

For Year Three, the Statement of Work as stated in the grant proposal included 2 tasks.

#8. Proposed in original grant: Control Program. Based on the results from the cost analysis results computed in the second year, we will institute a chlamydia control program for incoming basic recruits. If the mass therapy option is used, the effectiveness of it will be compared to a control group, which will consist of universally screened women with treatment of infected individuals, and which will be built into the study design.

Performed: The screening and treatment of female military recruits was continued.

Issues: Mass treatment was computed to be the most cost-effective intervention if the follow-up time was 5 years; however, the Fort Jackson IRB (Eisenhower, Ft. Gordon) denied approval to test such an intervention. The Fort Jackson IRB response is attached in Appendix 9.5. For this reason, continued screening of female recruits and treatment of positives was performed. Permission to have a mass treatment arm of the study was granted, however, by the Institutional Review Board (IRB) at Johns Hopkins. We plan to screen based on young age (≤ 25 years) the last year of the study.

#9. Proposed in original grant: Screening. We will continue to screen approximately 15,000 female recruits by urine LCR at Ft. Jackson to monitor prevalence. Treatment with azithromycin, 1.0 gram dose orally, will be offered to those who are infected with chlamydia.

Performed: There were 5657 females and 1203 males who were screened during the year.

Issues: Inability to schedule troops, due to troop commitment to other duties decreased the total number of female recruits screened over last year by 13.1%. We will start screening based on age in the fourth study year.

7 Conclusions and Future Plans

In view of results from the analyses described above and events over the past year, we are negotiating the following activities for the final year of this study. Each of these **six** point will be discussed further below.

- 1) continuation of the retrospective analysis of care obtained for PID in the women screened and those women not screened from January 1996 to September 1998 under the protocol described above;
- 2) re-specification of the CEA from a one-year analytic horizon;
- 3) continued screening of male recruits;
- 4) implementation of a screening protocol based on age equal to 25 or less for women in processing at Fort Jackson;
- 5) assessment of the impact of screening based on age on out-patient care obtained for PID in women offered screening and those not offered screening;
- 6) implementation of a questionnaire to obtain information on patient preferences for the use of self-administered swabs and possible use of the self-administered swab by a sub-set of volunteers at baseline.

Consistent with the protocol described and discussed above, we will continue to retrospectively document in-patient PID in women. Discussions amongst the investigators have recently determined that in the absence of data on sex mixing patterns after basic training, reinfection, and lack of longitudinal screening programs, conduct of the cost-effectiveness analyses from primarily a one-year analytic horizon, is most relevant to the goals of this study due to potential for re-infection. Consequently, the cost-effectiveness analysis will be conducted from a one-year analytic horizon which considers only PID prevented, as well as a multi-year horizon which considers PID, chronic pelvic pain, and ectopic pregnancies prevented, as has been done in the past.

Preliminary screening efforts for male recruits with internal review board (IRB) approval was conducted at Fort Jackson, SC from May 1998 to June 1998 identified a *C. trachomatis* prevalence

of 4.9%. Chlamydial infections in men directly impact re-infection rates in female recruits, limiting the ability of only one screening event to prevent sequelae in females. Documentation of the extent of infection in male recruits provides preliminary data to determine this potential of re-infection in women. The pilot data in men were based on 1,203 total men screened. To strengthen confidence in the prevalence estimates in male recruits, we will again screen for chlamydia in approximately 1,000 male recruits at Fort Jackson consistent with the protocol implemented during the spring of 1998. This project will be assisted by Dr. Jane Cecil, an infectious disease fellow at the Johns Hopkins University who will travel to Fort Jackson, SC. Arrangements for submission of a modified IRB application are in progress.

Consistent with the findings of the risk factor analysis noted above and documented in the recent New England Journal of Medicine publication, young age (i.e., age 25 or less) was the only factor available for practical use (i.e., facilitated access to data and high political feasibility) which was significantly associated (O.R. 3.0) with chlamydial infection in the female recruits screened at Fort Jackson, SC. Additionally, this screening strategy provides a cost-savings over no screening. Consequently, commencing in October 1998 we will alter the screening protocol to be consistent with the results of the analysis and screening in the PES will be limited to young women age 25 years or less. This strategy, which will provide screening for an estimated 87.9% of the incoming recruits, is expected to offer the potential to identify 95.3% of the infections in new female recruits.

Given that approximately 4.7% of all infections in new female recruits would not be identified by an age based screening protocol, we will conduct chart reviews to assess the magnitude of out-patient care for PID at Fort-Jackson in women who are screened and those not screened for the last year of the study. While this review is not intended to provide data for an exhaustive analysis of the impact of screening on PID, it will indicate potential impact on care sought within the first eight weeks of service. An addendum to the IRB to obtain informed consent to review records will be submitted for approval.

The impact of screening based on age on out-patient care obtained for PID in women offered screening and those not offered screening is currently being assessed. Hospitalizations (PASBA) for PID (ICD9 codes 614 and 615), infertility (ICD9 code 628), and ectopic pregnancy (ICD9 code 633) in Army enlisted females with less than or equal to one year of service are being examined per

person year for 1995, 1996, and 1997. Denominator data is being collected from the Defense Manpower Data Center, Monterey, CA. The Army recruits screened are being followed for hospitalization. Cases are being comprised of only those subjects that entered full-time active duty and exclude those going from basic training at Fort Jackson into the reserves who would not be hospitalized in the military healthcare system unless on temporary active duty. The remaining females entering the Army as enlisted soldiers during the same time period in which the cases were gained (as per DMDC) comprise the remainder of the cohort. The cohort is being followed for hospitalization for chlamydia sequelae through their first year of service.

Another Women's Defense Grant (P.I. Anne Rompalo, M.D.) being conducted at Fort Bragg, also a collaborative effort between Johns Hopkins and the Army, has found self-administered vaginal swabs (SAS) to be a potentially convenient alternative to the use of cervical and urine specimens with high sensitivity and specificity. Others have found the use of SAS to provide a sensitive and specific way to identify *C. trachomatis* in women which is highly acceptable ¹⁹. To assess the potential feasibility of future use of self-administered swabs we will implement a questionnaire to approximately 100 volunteers to determine the preferences of new female recruits with regard to screening test procedure, as well as ask them to provide a self-administered swab. We will compare prevalence obtained by urine to that from vaginal swabs in this sub-set of volunteers. An application for IRB approval is being drafted.

Urine-based screening for *C. trachomatis* by Ligase Chain Reaction was effective in a female military recruit population, as well as in a symptomatic Troop Medical Clinic population and an asymptomatic PAP clinic population. Acceptance was high, the urine specimens were readily obtained, and the assays were able to be performed quickly and efficiently.

The study has demonstrated a high prevalence (9.0%) for female recruits from a geographically and demographically diverse group, a substantial prevalence (11.9%) from a symptomatic Troop Medical Clinic population, and a higher than expected prevalence (7.3%) from an asymptomatic PAP clinic population. These results have indicated the need for an ongoing chlamydial control program in such female military groups.

Among recruits, risk factor analysis by multivariate logistic regression identified five independent, statistically significant, predictors for being infected with chlamydia: young age,

African American, vaginal intercourse, more than one new sex partner, and a new sex partner in the prior 90 days. Women that volunteered from the recruit population appeared to have behavioral characteristics that put them at high risk for chlamydial infections. Women, who were non-volunteers appeared to be similar demographically and many also practiced high risk behavior, but were significantly less likely to have these risk factors (prior chlamydia infection, vaginal sex, new sex partner, more than one sex partner, and inconsistent condom use) than were the volunteers.

Although the women from the symptomatic (TMC) and asymptomatic (PAP) groups had prevalences that were higher than the recruit population, their demographic and risk factor profiles were similar. The numbers of women enrolled at the present time are insufficient to perform univariate or multivariate regression analyses.

A chlamydial screening program that focused on screening all young female recruits (age 25 or less) would require that 87.2% of this population would be screened and 95.8% of all positive infections would be identified. Such a screening program as this, which employed urine LCR testing, has the potential to prevent pelvic inflammatory disease and ectopic pregnancy in Army women.

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9 Appendices

9.1 Articles published during the project's third year

9.1.1 Gaydos, C.A., Howell, M.R., Quinn, T.C., et al. 1998. Use of ligase chain reaction with urine versus cervical culture for detection of *Chlamydia trachomatis* in an symptomatic military population of pregnant and nonpregnant females attending Papanicolaou smear clinics. J Clin Microbiol. 36:1300-1304.

9.1.2 Kacena, K.A., Quinn, S.B., Howell, R.H., et al. 1998. Pooling urine samples for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. J Clin Microbiol. 36:481-485.

9.1.3 Gaydos, C.A., Howell, M.R., Pare, B., Clark, K.L., Ellis, D.A., Hendrix, R.M., Gaydos, J.C., McKee, K.T., and Quinn, T.C. 1998. *Chlamydia trachomatis* infections in female military recruits. New Eng Med J. 339:739-744.

Use of Ligase Chain Reaction with Urine versus Cervical Culture for Detection of *Chlamydia trachomatis* in an Asymptomatic Military Population of Pregnant and Nonpregnant Females Attending Papanicolaou Smear Clinics

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Received 13 November 1997/Returned for modification 27 January 1998/Accepted 16 February 1998

Ligase chain reaction (LCR) (Abbott Laboratories, Abbott Park, Ill.) with first-catch urine specimens was used to detect *Chlamydia trachomatis* infections in 465 asymptomatic military women attending clinics for routine Papanicolaou smear tests. Results were compared to results of cervical culture to determine the sensitivity of the urine LCR and the possible presence of inhibitors of amplification in pregnant and nonpregnant women. Discrepant results for LCR and culture were resolved by direct fluorescent antibody staining of culture sediments, two different PCR assays, and LCR for the outer membrane protein 1 gene. The prevalence of *Chlamydia* in specimens by urine LCR was 7.3% compared to 5% by culture. For 434 women with matching specimens, there were 11 more specimens positive by LCR than were positive by culture, of which all but one were determined to be true positives. There were four culture-positive, LCR-negative specimens, all from nonpregnant women. The sensitivity, specificity, and positive and negative predictive values of urine LCR after discrepant results were resolved were 88.6, 99.7, 96.9, and 99.0%, respectively. The sensitivity of culture was 71.4%. From the 148 pregnant women (prevalence by LCR, 6.8%), there were no patients who were cervical culture positive and urine LCR negative to indicate the presence in pregnant women of inhibitors of LCR. Additionally, a subset of 55 of the LCR-negative frozen urine specimens from pregnant women that had been previously processed in LCR buffer were inoculated with 5 cell culture inclusion forming units of *C. trachomatis* each and retested by LCR; all tested positive, indicating the absence of inhibitors of LCR in urine from these pregnant women. The use of LCR testing of urine specimens from asymptomatic women, whether pregnant or not, offers a sensitive and easy method to detect *C. trachomatis* infection in women.

Approximately 4 million *Chlamydia trachomatis* urogenital infections occur in the United States annually, and more than 50 million cases occur worldwide (7, 28). Unfortunately, symptoms are often mild or absent among infected men and women, leaving a large reservoir of infected persons to continue transmission to new sex partners (29). Chlamydial infections occur primarily among young sexually active persons. A high prevalence is common to all socioeconomic groups and may range from 5 to 20% in various groups of young adults (32, 33). Because of the high probability of progression of asymptomatic disease to serious sequelae, it has been recommended that individuals at risk for chlamydial infections be screened, especially women who are vulnerable to the serious consequences of genital infections, such as pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (7, 11). Urine can now be used to detect chlamydial infections in women by ligase chain reaction (LCR) (2, 8, 14, 20, 31, 34), which with its easily obtained specimen is a cost-effective method for screening programs for asymptomatic women (16). Because asymptomatic military populations have not been studied widely with

regard to chlamydial infections (4, 6, 10, 21, 26, 27) and because the sensitivity of the urine LCR assay has been reported to be low for samples from pregnant women due to the presence of inhibitors to amplification (18), we compared urine LCR to cervical culture for the detection of *C. trachomatis* in asymptomatic women attending clinics for routine Papanicolaou (PAP) smear tests.

MATERIALS AND METHODS

Populations and specimens. Military women ($n = 480$) attending clinics for a routine PAP smear test volunteered for a study to compare urine LCR tests to cervical cultures for the detection of *C. trachomatis* infections. The volunteer rate of the women approached by the civilian research nurse was 71%. The study was approved by the Institutional Review Boards of The Johns Hopkins University, the U.S. Army Medical Research Material Command, Fort Detrick, Frederick, Md., and Womack Army Medical Center, Fort Bragg, N.C. Of 480 women enrolled, 465 provided a urine specimen. All subjects completed a questionnaire for demographic information and behavioral risk factors for sexually transmitted diseases. The data instrument was a one-page, two-sided scannable bubble form (Scantron Corporation, Tustin, Calif.). During the pelvic examination, an endocervical swab was obtained by the attending clinician at the PAP smear clinic, who recorded clinical signs and symptoms on the data form. Culture swabs were placed into 2-sucrose-phosphate chlamydia transport medium. Commercial transport medium was replaced with in-house transport medium after 1 month of the study due to some toxicity of the former to tissue culture cells. Specimens were stored appropriately (4°C for urine specimens and -70°C for cultures) until shipping of the urine specimens at 4°C and cultures at -70°C. Shipments were made to ensure arrival at the laboratory within 4 days of collection. All specimens, consent forms, and data forms were shipped to Johns Hopkins Chlamydia Research Laboratory.

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Laboratory procedures. Urine specimens were processed and tested by LCR (Abbott Laboratories, Abbott Park, Ill.) according to the manufacturer's instructions. Briefly, 1 ml of urine was centrifuged at $15,000 \times g$ for 15 min. After the supernatant was removed, 1 ml of urine buffer was added to the pellet and the mixture was vortexed. After being heated at 97°C for 15 min, specimens were cooled and 100 μl of each specimen was added to an LCR unit dose tube. An appropriate chlamydia-positive control was included for the processing steps for each group of specimens. Additionally, two negative controls and two positive calibrator controls supplied by the manufacturer were used for each LCR assay run. After the amplification step in the automated thermocycler, unit dose tubes containing the specimens and controls were transferred to the automated enzyme immunoassay machine for the detection of amplified products. Tubes containing the amplified products were never opened; the automated enzyme immunoassay process sampled tubes by piercing the tops of the unit dose tubes, which prevented amplicon contamination. In order to prevent other sources of contamination, specimens were processed in a designated room separate from the room used to amplify and detect specimens. Gloves were frequently changed and aerosol-barrier pipette tips and dedicated pipettors were used. Strict quality-control measures such as machine maintenance checks, daily cleaning of laboratory areas and equipment with bleach, and area swipe tests to monitor amplicon contamination were employed.

Culture specimens were stored frozen at -70°C for up to 3 days. Cultures were done in 96-well microwell plates in McCoy cells by standard methods (12). Tissue cultures were stained with genus-specific fluorescein-conjugated antibody (Kallested, Chaska, Minn.) and species-specific antibody (Boehringer Mannheim/Syva, San Jose, Calif.). Stained cultures were read for the presence of chlamydial inclusion bodies with an epifluorescence microscope.

Discrepancy analysis was done for any sample with discordant results between culture and LCR. A sample that was positive by culture and negative by LCR was considered to be a true positive, but the discrepancy was investigated for the presence of inhibitors to amplification by LCR. The urine LCR was repeated from the originally processed specimen and repeated again after diluting the processed specimen 1:10 in urine LCR buffer to check for the presence of inhibitors in the specimen. (Dilution has been shown to sometimes decrease the concentration of the inhibitor enough to allow a true-positive specimen to be amplified.) Additionally, PCR (Roche Diagnostic Systems, Branchburg, N.J.) was done on an archived aliquot of frozen urine and another LCR was done for a different DNA target, the outer membrane protein 1 (OMP-1) gene. For specimens that were positive by LCR and negative by culture, the culture specimen transport sediment was stained by direct fluorescent antibody (DFA) (Boehringer Mannheim/Syva) for chlamydial elementary bodies. PCR also was done on the specimens from the culture transport vials. In addition, PCR was done on the archived urine and an LCR for the OMP-1 gene was done on the previously processed (buffered) urine specimen. Specimens that were positive by one or more of the ancillary tests were considered true positives. An LCR-positive urine specimen which could not be confirmed by another test was considered to be a false positive.

Testing of urine specimens from pregnant women. A subset of all available ($n = 55$) previously processed (buffered) LCR-negative urine specimens that were from pregnant women were inoculated with 5 inclusion forming units of *C. trachomatis* and retested by LCR to check for the presence of inhibitors. Additionally, 65 archived LCR-negative unprocessed urine specimens that were available from pregnant women were tested by a research internal control assay to evaluate the presence of inhibitors (9). This assay tested for the ability to amplify an extraneous sequence of DNA which was added as an internal control to the specimen. The assay contained primers for the extraneous DNA internal control as well as the primers for the organism of interest. A positive amplification of the internal control indicated that the specimen contained no inhibitors to the amplification process, while a negative result indicated that the specimen contained something which inhibited the amplification process.

Data analysis. The data from the questionnaire forms were scanned into a data set (D-base III Plus; Ashton Tate, Borland International, Spring Valley, Calif.), and LCR results, demographics, and risk factor information were analyzed by the chi-square test, Fisher's tests of exactness, and univariate analysis (Intercooled Stata, version 4.0; Stata Corporation, College Station, Tex.).

RESULTS

Patient characteristics. Among the 480 women enrolled, only 1 woman had reported mild symptoms and the remainder were asymptomatic. Approximately half (55.2%) were 25 years or younger, and 50.8% were African-American. Over 90% were enlisted personnel, 98.3% reported vaginal sex, 11.3% had a new sex partner in the previous 90 days, 15.2% had more than one sex partner in the previous 90 days, 88.5% reported inconsistent condom use, and 30.8% were pregnant (Table 1). Reasons for clinic visit, clinical presentation, and sexual risk history are presented in Table 1. Of the 465 women who provided a urine specimen, the overall prevalence for chla-

TABLE 1. Characteristics of military women screened for *C. trachomatis* at Fort Bragg, N.C.

Variable	No. of women ($n = 480$)	%
Median age, yr (range) ^a	25 (19-47)	
Ethnicity ^b		
White	181	37.7
African-American	244	50.8
Other (American Indian, Alaskan, or Asian Pacific)	52	10.8
Military category ^c		
Enlisted personnel	439	91.5
Officer	36	7.5
Reason for clinic visit ^d		
Sex partner of infected individual	1	0.2
Complaint of symptoms	1	0.2
Screening	470	97.8
Other	4	0.8
Clinical presentation ^d		
Mucopus	1	0.2
Cervicitis	5	1.0
Ectopy	5	1.0
Cervical motion tenderness	3	0.6
Friability	39	8.1
Pregnant	148	30.8
Normal exam	348	72.5
Sexual history (past 90 days)		
More than one sex partner	73	15.2
New sex partner	54	11.3
Consistent condom use	51	10.6
Inconsistent condom use	425	88.5
Previous diagnosis ^e		
<i>N. gonorrhoeae</i>	20	4.2
<i>C. trachomatis</i>	86	17.9
Syphilis	5	1.0
Trichomonas	41	8.5
None	348	72.5
Chlamydia-positive urine LCR ^f	34	7.3
Chlamydia-positive culture ^g	24	5.3

^a Data missing for eight women (1.7%).

^b Data missing for three women (0.6%).

^c Data missing for five women (1.0%).

^d Data missing for 27 women (5.6%).

^e Data missing for two women (0.4%).

^f Urine specimen for LCR missing for 15 women.

^g Cervical specimens for culture missing for 21 women and 10 toxic specimens collected.

mydia infection by LCR was 7.3%. The prevalences of infection for other categories based on LCR included 11.0% for women ≤ 25 years of age, 8.9% for African-American women, and 6.8% for pregnant women. By risk category the prevalences were 15.1% for those with a new sex partner in the previous 90 days, 10.3% for those with more than one sex partner in the previous 90 days, 7.5% for those with inconsistent condom use, 7.4% for those reporting vaginal sex, and 3.6% for those with a prior chlamydial infection.

In univariate analysis only young age (≤ 25 years) (odds ratio [OR], 4.23; 95% confidence interval [CI], 1.72 to 10.43) and a new sex partner (OR, 2.61; 95% CI, 1.11 to 6.1) were predictors of chlamydial infection (Table 2). However, when we controlled for age, a new sex partner was no longer significant.

Comparison of urine LCR to cervical culture. Of the 465 women, 31 women did not have matched culture specimen results. Ten specimens were toxic for tissue culture and no cervical cultures were collected from 21 women, leaving 434 matched specimens for comparison. After the use of the commercial chlamydia transport buffer was stopped and the in-

TABLE 2. Univariate analysis of results relative to factors associated with positive urine LCRs for military women attending PAP smear clinics

Factor ^a	% with a positive LCR		OR (95% CI)	P
	Factor absent	Factor present		
Age ≤25 yr (254)	2.8	11.0	4.2 (1.72, 10.43)	0.002
African-American (233)	6.8	8.6	1.3 (0.61, 2.7)	0.501
Pregnant (142)	7.4	7.0	0.94 (0.44, 2.03)	0.882
Normal pelvic exam (275)	12.5	6.6	0.49 (0.18, 1.31)	0.154
Prior diagnosis of STD ^b (127)	8.3	3.9	0.45 (0.17, 1.2)	0.110
Having had more than one sex partner in last 90 days (68)	6.8	10.3	1.6 (1.53, 3.73)	0.316
Having had a new sex partner in last 90 days (53)	6.4	15.1	2.6 (1.11, 6.10)	0.027 ^c

^a Numbers in parentheses represent numbers of individuals with the factor present ($n = 465$).

^b STD, sexually transmitted disease (chlamydia, gonorrhea, syphilis, or trichomonas).

^c A new sex partner was not significant when we controlled for age.

house 2-sucrose-phosphate medium was used, no further specimens toxic to tissue culture were observed. Among the 31 specimens without matched results, there were two LCR-positive urine specimens for which a matching cervical culture was not collected.

From the 434 matched specimens, 32 (7.4%) were LCR positive, of which 31 (7.3%) were confirmed as true positives (Table 3). There were 21 LCR-positive, culture-positive specimens. Four patients had urine-LCR-negative, cervical-culture-positive specimens. Discrepancy analysis of these LCR-negative, culture-positive specimens demonstrated that one was positive in the repeat LCR assay and was OMP-1 LCR positive, one had a negative value which was close to the cutoff value for a positive result and was PCR positive when the archived frozen urine was tested, one had a culture transport specimen that was PCR positive, and the results of one could not be confirmed by any of the ancillary tests, including repeat culture. The initial LCR-negative results from these four urine specimens were all considered to be false negatives.

There were 11 specimens that were LCR positive and culture negative, 10 of which could be confirmed as true-positive specimens (Table 4). Five were DFA positive, six were urine PCR positive, seven were culture PCR positive, and eight were OMP-1 LCR positive. Thus, all but one of these LCR-positive specimens were confirmed as true positives by at least one or more additional assays. After resolution of the discrepant results, the sensitivity, specificity, and positive and negative predictive values of urine LCR were 88.6, 99.7, 96.9, and 99.0%, respectively (Table 3), and the sensitivity of culture was 71.4%.

TABLE 3. Comparison of urine LCR to cervical culture for *C. trachomatis* in military women attending PAP smear clinics

Test	Result	No. of women ^a with test result (%)	Resolved patient infection status ^b	
			Positive	Negative
Cervical culture	Positive	25 (5.8)	25	0
	Negative	409 (94.2)	10	399
Urine LCR ^c	Positive	32 (7.4)	31	1
	Negative	402 (92.6)	4	398

^a Four hundred eighty women enrolled; 434 had matching specimens.

^b Resolved patient infection status was defined as the resolved status of a patient with a positive culture or with a urine specimen positive by two tests (LCR, DFA staining, PCR OMP-1 gene, PCR plasmid gene, and LCR OMP-1).

^c After discrepant results were resolved, sensitivity was 88.6%, specificity was 99.7%, and positive and negative predictive values were 96.9% and 99.0%, respectively (sensitivity of culture, 71.4%).

LCR of urine of pregnant women. There were 148 urine specimens from pregnant women. The prevalence of chlamydia infection by LCR for the pregnant women was 6.8%, and that for the nonpregnant women was 7.8%. There were no culture-positive, LCR-negative results from pregnant women which could have indicated the presence of LCR inhibitors. All four of the culture-positive, LCR-negative specimens were from women who were not pregnant. In addition, a subset of 55 LCR-negative urine specimens, previously processed in LCR buffer and frozen, which were from pregnant women and were inoculated with chlamydia and retested by LCR were all LCR positive, indicating the lack of inhibitors. Of the 65 available archived urine specimens from pregnant women which were LCR negative and tested in the internal control assay, there were 3 (4.6%) that exhibited inhibition based on a negative value for amplification of the internal control.

DISCUSSION

Chlamydia infections were of a higher prevalence than expected from these asymptomatic military women attending a clinic for a routine PAP smear test. An LCR prevalence of 7.3% underscores the necessity for the recommendation to screen all sexually active young women when they are attending a routine health care clinic (7). The high prevalence of 11.0% for those ≤25 years of age confirm the result of studies of others that young age is a significant risk factor for chlamydial infections (13, 17, 22). These results indicate the need

TABLE 4. Resolution of urine-LCR-positive and cervical-culture-negative discrepant results for *C. trachomatis* in military women attending PAP smear clinics ($n = 11$)

Laboratory no.	Test result					Status
	LCR (urine)	DFA ^a	PCR (urine)	PCR (cervix)	LCR for OMP-1 (urine)	
1264	+	—	—	—	+	Confirmed
2407	+	+	+	+	+	Confirmed
3197	+	+	+	+	+	Confirmed
3659	+	—	—	+	—	Confirmed
3891	+	—	—	—	+	Confirmed
5560	+	+	—	+	+	Confirmed
5570	+	—	—	—	—	Unconfirmed
6082	+	—	+	+	+	Confirmed
6280	+	+	+	+	—	Confirmed
6966	+	+	+	+	+	Confirmed
8016	+	—	+	—	+	Confirmed

^a DFA staining of culture transport vial specimen.

for an ongoing chlamydial control program for such female military personnel as those enrolled in this study. This population demonstrated a high degree of sexual behaviors placing them at risk for sexually transmitted diseases, with 98% being sexually active, 15% having more than one partner, 11% having a new partner in the last 90 days, and 88% using condoms inconsistently. All of these behaviors have been shown by others to be predictive of chlamydial infection (1, 22–24, 36). In the univariate analysis for this study, both young age (prevalence, 11.0%) and having had a new partner (prevalence, 15.1%) reached statistical significance. However, when we controlled for age, a new sex partner was not significant. Young age (≥ 25 years), which is an easily determined risk factor and which is a nonthreatening question for those women who may be reticent to answer questions about their sexual behavior, appears to be an excellent predictor of chlamydia infection and can be recommended for deciding who should be screened in clinical or outreach situations (13, 17).

Urine LCR performed well in this study of asymptomatic women, with a sensitivity of 88.6%, which is similar to that demonstrated by others for asymptomatic women (87.5%) (2). Compared to cervical culture, which had a sensitivity of 71.4%, LCR detected more infected women. Many reasons can account for the lower culture sensitivity. Not only can the cold chain of transport be interrupted, but the quality of the transport medium is important as well. Initially, a commercially available transport medium was used in this study, which resulted in many (10) toxic tissue culture results. Quality-control assays of the remaining lot of uninoculated transport medium demonstrated that it was toxic to cells in tissue culture. After switching to the use of our own transport medium, which is quality controlled in tissue culture, we observed no further toxicity.

Additionally, the quality of the endocervical specimen, as measured by the presence of columnar epithelial cells, has been shown to play a significant role in the numbers of positive specimens (19, 37). In another study of family-planning clinics in Baltimore, Md., clinicians obtained adequate specimens only 72.3% of the time (37). Thus, inadequate cervical swab specimens could have contributed to the lower sensitivity of culture in our study. Other studies have demonstrated higher sensitivities for urine LCR than cervical culture (2, 5, 8, 20, 31, 34). Sensitivities for cervical culture in these studies has ranged from 45.5 to 46.9% to 55.6 to 65.0% (2, 5, 8, 34). Schachter et al. have demonstrated that the sensitivity of culture for *C. trachomatis* may be increased from 67.1% to 74% by adding a urethral swab culture, which could be indicative that some women may be infected only in the urethra and not the cervix (31). This could help explain the higher number of positives found by urine LCR, presumably reflecting infections from both the cervix and the urethra. Because urine is an easy-to-obtain, noninvasive specimen giving accurate results with LCR, it is ideal for screening asymptomatic individuals who may not be presenting for a pelvic exam or for outreach screening programs.

Although our study enrolled only 148 women who were pregnant, we did not observe any indication of inhibitors in urine specimens, as evidenced by the lack of urine-LCR-negative results when the cervical culture was positive. Although there were four such specimens in this study, they were all from nonpregnant women. Another study has reported a significant problem with inhibitors in urine with use of the LCR test; however, the urine specimens were transported at ambient temperatures, which may have influenced the LCR results (18, 25, 30). The spiking experiment in our study did not demonstrate any inhibitors in the 55 LCR-negative, previously frozen

urine specimens from pregnant women. It is possible that freezing and thawing of these processed urine specimens reduced or destroyed some LCR inhibitors. Freezing and thawing reduced the inhibition from 19 to 16% in one study (35). Additionally, the experiment which tested the archived urine of 65 pregnant women demonstrated only three (4.6%) inhibited specimens. This value is of the same order of magnitude as that reported by others for inhibition in urine specimens (2.6 and 1.8%) for amplified testing (3, 15). Most investigators now believe that inhibitors to amplification exist for both urine and cervical specimens (3, 15, 35). A combination of heat treatment (95°C for 10 min) and 10-fold dilution of the processed specimen reduced inhibition of PCR from 19 to 4% in one study (35). The pH of the cervical mucosa was partly correlated with inhibitors (35). Decreased inhibition was found at pH values of ≥ 7.5 . The degree to which inhibitors to amplification influence the prevalence detected by LCR and PCR needs to be further studied. Roche Molecular Systems has addressed this problem by incorporating an internal DNA control amplification and detection assay into their new combination PCR assay for *C. trachomatis* and *Neisseria gonorrhoeae*, which will prove to be a great advance in the diagnostic capability of amplification assays. Specimens exhibiting inhibitors can be diluted or heated and their DNA can be extracted, and tests can be repeated. The use of the internal control will give a greater degree of confidence to the validity of a negative amplification result. Consideration of the use of an internal control should be given for amplification tests in the future. The College of American Pathologists now requires examination of a control to assess the presence of inhibitors in all amplification procedures.

In summary, young sexually active women, including those in the military, should be frequently screened for chlamydia infections. Urine LCR offers an easy and sensitive method to accomplish this, especially for women not presenting for a pelvic examination. It is cost-effective in preventing the expensive sequelae of pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (16).

ACKNOWLEDGMENTS

We thank the study coordinator, Barbara Pare; the research nurses Eleanor Howard, Katy Cline, and Bobbi Jones and the staffs of the Fort Bragg clinical sites for obtaining specimens; the laboratory technicians Graciela Jaschek, Laura Welsh, Dien Pham, Diana Perkins, Sandy Leister, and Kimberly Crotchfield for performance of laboratory tests and data entry; Kathryn Clark for statistical assistance; and Pat Buist for assistance in manuscript preparation.

Funding for this study was from Department of the Army grant DAMD 17-95-1-5064.

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Pooling Urine Samples for Ligase Chain Reaction Screening for Genital *Chlamydia trachomatis* Infection in Asymptomatic Women

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Received 23 July 1997/Returned for modification 19 September 1997/Accepted 4 November 1997

The accuracy of pooling urine samples for the detection of genital *Chlamydia trachomatis* infection by ligase chain reaction (LCR) was examined. A model was also developed to determine the number of samples to be pooled for optimal cost savings at various population prevalences. Estimated costs included technician time, laboratory consumables, and assay costs of testing pooled samples and retesting individual specimens from presumptive positive pools. Estimation of population prevalence based on the pooled LCR results was also applied. After individual urine specimens were processed, 568 specimens were pooled by 4 into 142 pools and another 520 specimens were pooled by 10 into 52 pools. For comparison, all 1,088 urine specimens were tested individually. The sample-to-cut-off ratio was lowered from 1.0 to 0.2 for pooled samples, after a pilot study which tested 148 samples pooled by 4 was conducted. The pooling algorithm was 100% (48 of 48) sensitive when samples were pooled by 4 and 98.4% (61 of 62) sensitive when samples were pooled by 10. Although 2.0% (2 of 99) of the negative pools of 4 and 7.1% (1 of 14) of the negative pools of 10 tested presumptive positive, all samples in these presumptive-positive pools were negative when retested individually, making the pooling algorithm 100% specific. In a population with 8% genital *C. trachomatis* prevalence, pooling by four would reduce costs by 39%. The model demonstrated that with a lower prevalence of 2%, pooling eight samples would reduce costs by 59%. Pooling urine samples for detection of *C. trachomatis* by LCR is sensitive, specific, and cost saving compared to testing individual samples.

There are 89.9 million cases of genital *Chlamydia trachomatis* infection every year worldwide (13), 4.5 million of which occur in the United States (4). Although many *C. trachomatis* infections are asymptomatic (16), the sequelae from infection, including pelvic inflammatory disease (PID), and infertility, represent a large burden for populations worldwide. Furthermore, inflammatory sexually transmitted diseases, such as those caused by *C. trachomatis*, increase the risk of both human immunodeficiency virus (HIV) transmission and infection (7, 11). Together, the high percentage of asymptomatic infections, the sequelae of infections, and the increased association with HIV transmission underscore the importance of screening as a necessary intervention to reduce the burden of diseases caused by *C. trachomatis*.

Detection of genital *C. trachomatis* infection by ligase chain reaction (LCR) with first-void urine is a noninvasive, highly sensitive, and highly specific procedure (2, 8). Although the cost of LCR is higher than that of other tests such as direct fluorescent antibody, antigen detection by enzyme immunoassay, and nucleic acid probe tests, LCR is more sensitive and more specific (15, 17). Culture has been considered to be the "gold standard" in the past but costs more and is less sensitive than either LCR or PCR (3, 5, 10, 12, 14).

Pooling serum samples for HIV testing was found to be

accurate and has been used to reduce the cost of enzyme-linked immunosorbent assays for detection of antibody to HIV (1, 6). Pooling for HIV testing has been used to develop both population estimates and, in a multiple-step procedure, to determine which individual sample is positive. Pooling has also been applied to the PCR detection of *C. trachomatis* in endocervical and urethral scrapes (9), but in that study the sample size was small. The investigators acknowledged the need for subsequent studies to rule out the possibility of reduced sensitivity by diluting out individual specimens in the pool.

The screening of women at risk for *C. trachomatis* has been recommended by the Institute of Medicine as a cost-effective program which would prevent the high cost of untreated infections which lead to PID (4). As a screening and treatment intervention reduces the prevalence of *C. trachomatis* infection over time, the cost per specimen tested with the pooling protocol algorithm would be further decreased. The reduction in price occurs for two reasons: (i) as prevalence decreases, pooling a greater number of samples increases cost savings and (ii) the samples from fewer pools would test presumptive positive such that fewer samples would be retested individually. Therefore, the cost for finding one case does not increase dramatically as prevalence decreases, as is the case when samples are tested individually.

In this study we examined the accuracy and cost-saving ability of pooling urine specimens for the detection of genital *C. trachomatis* infections by LCR. A cost analysis of the pooling protocol was conducted to determine the number of specimens it would be necessary to pool in order to provide the

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highest cost savings, taking into account the prevalence of infection in the population screened.

MATERIALS AND METHODS

Sample size and parameters. As part of an ongoing study to determine chlamydia prevalence in asymptomatic U.S. Army females with a mean age (\pm standard deviation [SD]) of 22 (± 4 years), urine samples were tested by LCR to ascertain genital *C. trachomatis* infection. A sample of 568 processed urine specimens was pooled by 4 into 142 pools, and 520 specimens were pooled by 10 into 52 pools. Pools were formed by order of consecutive laboratory accession number. All 1,088 pooled urine samples were also tested individually. For all discrepant individual and pool results, both the individual samples and the pools were retested to confirm results.

Urine specimen, collection, preparation and assay setup. Specimen collection, preparation, and assay setups were performed according to the manufacturer's instructions for the urine-based chlamydia LCR assay (Abbott Laboratories, Abbott Park, Ill.).

Specimens were refrigerated immediately after collection and shipped overnight delivery with wet packs to maintain refrigerator temperature. Specimens were either processed immediately on arrival at the laboratory or refrigerated and processed within 2 days. The total time before processing never exceeded 4 days as per the LCR package insert. Processed refrigerated specimens were amplified the day after processing. Processed urine can be refrigerated or frozen for up to 60 days before testing. We refrigerated our processed specimens for up to 7 days in case retesting was needed.

One milliliter of urine was centrifuged at $\geq 9,000 \times g$ for 15 min (± 2 min) at room temperature. The supernatant was removed, and the pellet was resuspended into 1.0 ml of LCR urine specimen resuspension buffer and vortexed. Preparations were then boiled at 97°C (± 2 °C) for 15 min (± 1 min) to extract the DNA and stored at 2 to 8°C for up to 7 days until tested. Processed urine specimens were subsequently tested individually and tested pooled.

When specimens were tested individually, a volume of 100 μ l of processed urine specimen was placed into its own LCR chlamydia amplification vial (unit dose). For each pool of four, 25 μ l of each of the four processed specimens was placed into a single unit dose. For each pool of 10, 10 μ l of each of the ten processed specimens was placed into a single unit dose. The total specimen volume was then 100 μ l for each unit dose. Two negative controls, two positive calibrators, and a positive processing control were included in every amplification run in accordance with the manufacturer's instructions.

DNA amplification and detection. Unit dose tubes containing DNA preparations were amplified under the following conditions: 40 cycles of denaturation at 93°C for 1 s, annealing at 59°C for 1 s, extension at 62°C for 1 min, 10 s, and soaking at 25°C in an LCR thermocycler (Abbott Laboratories). Amplified DNA was detected in an LCR-automated machine which performed a particle-based enzyme immunoassay with a fluorescent signal. For individually tested samples, a sample-to-cutoff ratio (S/CO) of ≥ 1.0 was considered positive, and borderline negative samples (0.80 to 0.99 S/CO) were retested, according to manufacturer's instructions.

Pilot study. Because the volume for each individual urine specimen is decreased in the pooled assay, a pilot study was conducted to determine an appropriate S/CO for the pooled assays. The desired S/CO would detect all positive pools while not detecting most, if not all, negative pools. The pilot study consisted of 148 processed urine samples from the ongoing study of female U.S. Army recruits. The technician, blinded to the individual test results, pooled and tested these 148 samples by four. By lowering the S/CO from 1.0 to 0.2, all of the positive pools were detected (100%) (25 of 25) and only 2.7% (1 of 37) of the negative pools tested presumptive positive. Since all pools which test positive are retested, specificity with the pooling algorithm is 100%, i.e., no different than with testing processed specimens individually.

Cost analysis. A model was developed to determine the pool size that yielded the highest cost savings. The binomial distribution was used to estimate the number of pools that are likely to be positive given a selected pool size and population disease prevalence. Next, the optimal pooling number for a range of disease prevalences was calculated. For a dichotomous outcome (i.e., positive or negative test result for a genital *C. trachomatis* infection), independence was assumed (i.e., the order of the samples received was random with regard to the distribution of the positive or negative samples in the population). The expected percentage of positive pooled assays was determined using the following equation: $s = [(1 - r/n)^c] \times 100\%$, where s is the expected number of positive pools, r is the number of positive samples tested, n is the total number of samples tested, r/n is the prevalence of disease, and c is the number of specimens pooled. This equation accounted for the probability that from 1 to c samples in the pool were positive.

A baseline total cost of \$12.76 per individual sample which included \$0.36 for laboratory consumables, \$3.56 for technician cost, and \$8.84 for the LCR assay was used. Laboratory consumables include gloves and supplies used for handling samples. Technician cost was calculated assuming an average of 10 runs per week (i.e., 380 samples), an annual salary of \$30,000 with an additional 28% of salary in benefits, and a 69% laboratory or university overhead (i.e., $\$30,000 \times 1.28 \times 1.69 = \$64,896$). The cost of the five controls used for each 19 specimens tested was calculated into the LCR assay cost, in which the base cost per unit dose was

\$7. A sensitivity analysis was also done first, with the base cost per unit dose ranging from \$5 to \$15, technician cost set at \$3.56, and cost for laboratory consumables set at \$0.36 per specimen tested. In the second sensitivity test, the annual salary of the technician ranged from \$20,000 with 20% overhead to \$40,000 with 69% overhead, unit dose cost was set at \$7.00, and cost for laboratory consumables set at \$0.36 per specimen tested. Low technician and low assay costs as well as high technician and high assay costs were also calculated.

Estimation of population prevalence with pooled data. Pooling can also be used to reduce the cost of estimating population prevalence. Based on calculations from a previous study, the estimated population prevalence and 95% confidence interval (CI) were back calculated from the pooled data (6). Separate estimates were made for samples pooled by 4 and by 10. Calculations were based on the following equations: (i) Estimated prevalence: $p = 1 - [1 - (s/n)]^{1/c}$ (ii) (SD): $SD = \{[(s/n) \times (1 - s/n)^{(2/c)}] / [(n \times c^2)]\}^{0.5}$ (iii) 95% CI: $p \pm 1.96$ (SD) where s is the total number of presumptive-positive pools, n is the total number of pools, and c is the number of specimens in each pool.

RESULTS

Sensitivity and specificity of the pooled assays. A comparison of the distribution of S/COs for the individual and pooled samples indicated that lowering the S/CO from 1.0 to 0.20 for determining positive pools resulted in high sensitivity with a low proportion of specimens from negative pools that need to be retested individually (Fig. 1). There were two weakly positive individual specimens (i.e., an S/CO of ≥ 1 but < 2.0) in the pilot study (pooled by 4), six weak positives in the study pooled by 4, and four low positives in the study pooled by 10. These weak positives were the only positive specimens in the pool. These pools all tested between 0.2 and 1.0 and sometimes higher.

The pooling algorithm was 100% (48 of 48) sensitive when pooling by 4 and 98.4% (61 of 62) sensitive when pooling by 10 (Table 1). Although 2.0% (2 of 99) of the negative pools of four and 7.1% (1 of 14) of the negative pools of 10 tested presumptive positive, all of the samples in these pools would be retested individually, according to the pooling algorithm. Retesting the individual samples in the presumptive-positive pools resulted in no false-positive specimens (100% specificity).

Cost analysis. For a population with 8% genital *C. trachomatis* prevalence, which is close to the 8.5% prevalence found in our study population of female U.S. Army recruits, pooling by four provided the highest cost savings. The reduction of total assay costs per specimen, which included technician time, decreased from \$12.76 to \$7.78, i.e., by 39%. The model demonstrated that with a 2% prevalence, pooling eight samples would reduce the cost per sample by 59%. A population prevalence graph was constructed from the model to determine the number of pooled samples that would achieve the highest cost savings (Fig. 2).

A sensitivity analysis for the cost savings model was conducted with ranges of both technician and LCR unit dose costs. For specimens tested individually, raising the base cost of the LCR unit dose from \$7 to \$15 resulted in an increase of the total cost per specimen tested from \$12.76 to \$22.87, whereas lowering the cost of the LCR unit dose to \$5 reduced the total cost per specimen tested to \$10.24. Similarly, raising the annual salary of the technician from \$30,000 to \$40,000 and assuming 28% benefits and 69% overhead increased the total cost per specimen tested from \$12.76 to \$13.95, whereas lowering the technician's annual salary to \$20,000 and the overhead to 20% reduced the total cost per specimen tested from \$12.76 to \$10.89. The cost per specimen tested with the low unit dose and low technician costs was \$11.43, while the high unit dose and technician costs yielded a cost of \$24.06 per specimen tested.

For a population prevalence of 8% and pooling by four, raising the unit dose cost from \$5 to \$15 would result in a total

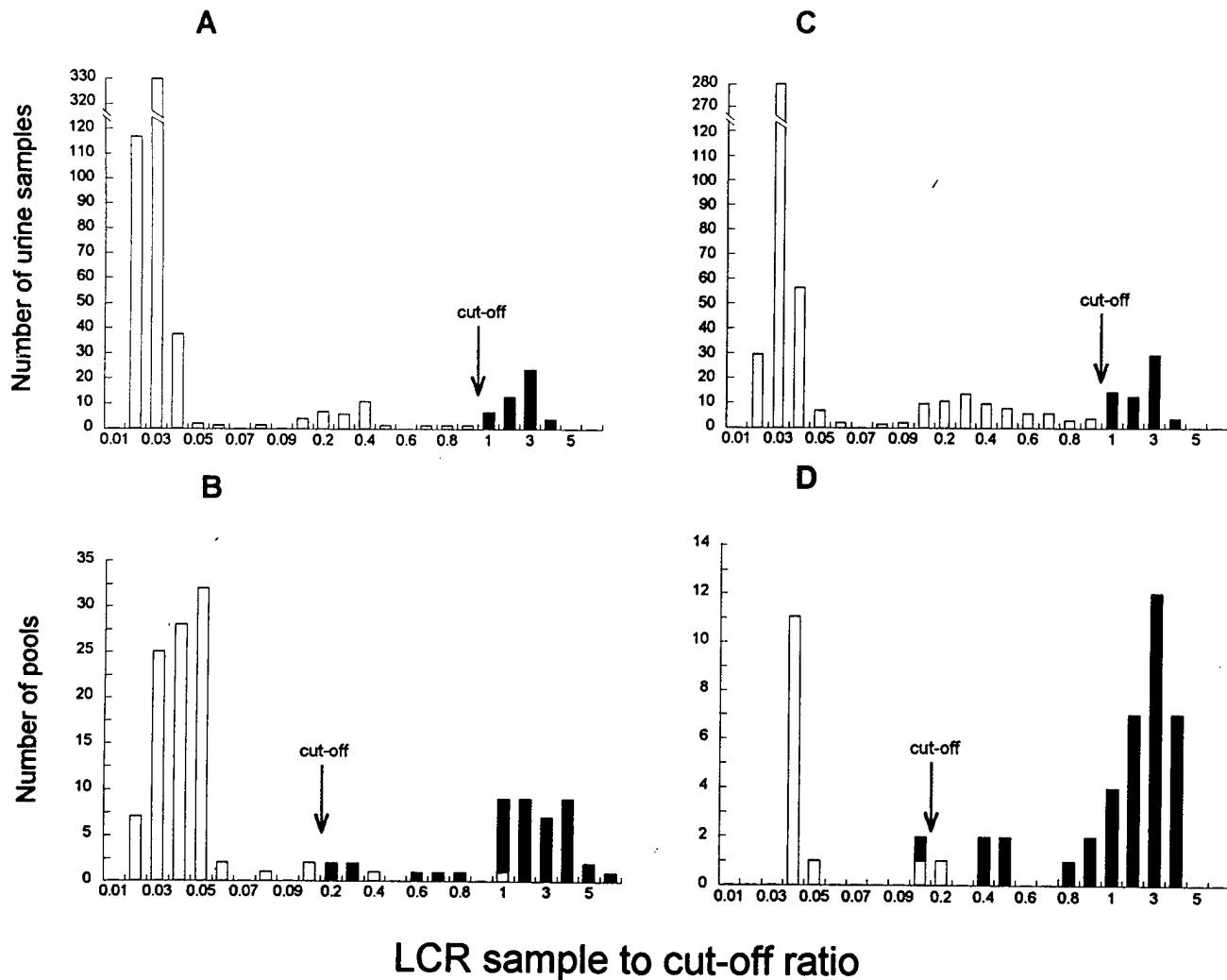


FIG. 1. Detection of genital *C. trachomatis* infection by LCR. Graphs A and C show the distribution of individual urine samples from two groups of 568 and 520 women taken from the study population. Graph B shows the distribution of the samples in A pooled by 4 ($n = 142$), and graph D shows the distribution of the samples in C pooled by 10 ($n = 52$). The S/CO for individual samples, which was 1.0, was lowered to 0.2 for pooled samples, as indicated.

cost of \$6.43 and \$13.17, respectively, per specimen tested. Ranging technician cost from low to high would result in a total cost increase of \$6.36 and \$8.68, respectively, per specimen tested. The overall savings of the pooling algorithm over individual testing ranged from 37 to 42% when low to high unit dose cost was considered. Similarly, the overall savings ranged from 42 to 38% when a low-to-high technician cost was considered. The total cost per specimen with the pooling by four algorithm with both the low unit dose and low technician cost was \$5.01 per specimen tested, and that with the high unit dose and high technician cost was \$14.07 per specimen tested. In all of these scenarios, pooling provided a cost savings compared with individual testing.

Estimation of population prevalence with pooled data. The observed prevalence for the individual samples in the 142 pools of 4 was 8.5% (48 of 568), and that for the 52 pools of 10 was 11.9% (62 of 520) (Table 1). The estimated population prevalence, back calculated from the number of positive pools, for the 142 pools of 4 was 9.1 (95% CI: 6.5 and 11.6), and for the 52 pools of 10 it was 12.9 (95% CI: 8.8 and 17.0). Each 95% CI included the observed prevalence of the subsample, 10.1% (110 of 1,088). Additionally, each 95% CI included values

within 8 to 9%, the overall prevalence measured in a much larger sample (>10,000) of this population.

DISCUSSION

In this study we evaluated pooling of processed urine specimens for LCR detection of *C. trachomatis* for both accuracy and cost-saving ability. The high sensitivity and specificity of LCR was not affected by pooling up to 10 samples when the S/CO was adjusted from 1.0 to 0.2. Although a small percentage of negative pools tested presumptive positive, no specificity was lost with the pooling algorithm, since all specimens in pools which test presumptive positive are retested individually with the manufacturer's specified S/CO for the individual test. Since retesting negative pools does increase costs, the specificity of pools must be high.

The cost analysis model showed that depending on the prevalence of *C. trachomatis*, the number of specimens that should be pooled for optimal cost savings varies. As prevalence decreases, the pooling protocol for screening could save more than 59% of the cost per specimen compared to that for testing individual samples only. Also, early studies have shown that *C.*

TABLE 1. Accuracy of pooling urine samples for the detection of genital *C. trachomatis* infection in asymptomatic women by LCR^a

Parameter	Result for pool size with indicated no. of samples	
	4	10
Total no. of urine samples	568	520
No. of positive specimens (%)	48 (8.6)	62 (11.9)
No. of pools	142	52
No. of presumptive-positive pools	45	39
Estimated population prevalence calculated from pooled data (95% CI)	9.1 (6.5, 11.6)	12.9 (8.8, 17.0)
No. of samples retested individually (%)	180 (31.7)	390 (75.0)
No. of positive pools (%)	43 (30.3)	38 (73.1)
Sensitivity of the pools (%)	43/43 (100)	37/38 (97.4)
Pooling algorithm sensitivity (%)	48/48 (100)	61/62 (98.4)
Specificity of the pools (%)	97/99 (98.0)	13/14 (92.9)
Pooling algorithm specificity (%)	520/520 (100)	458/458 (100)
Total no. of assays performed	322	442
No. of assays saved	246	78

^a For the pooling algorithm, samples were first tested pooled and then presumptive-positive pools were retested individually.

trachomatis screening and treatment programs are cost effective; the Centers for Disease Control and Prevention has estimated that for every dollar spent on prevention, \$12 is saved in treating sequelae (4). The use of the pooling algorithm for testing samples obtained during screening could further increase savings in health care costs.

Since *C. trachomatis* prevalence levels have ranged from 4 to 20% in various populations in the United States, pooling three to four samples is likely to provide the highest cost savings. Furthermore, the cost saved does not significantly change the sensitivity or specificity of the assay. In the event that screening is not conducted, pooling can be used to determine population prevalences over time in order to measure the benefits of disease interventions such as mass treatment or behavioral interventions. The population prevalence back calculation, described previously (6), gave an accurate estimate of the observed population prevalence in this study.

Use of the pooling algorithm would benefit investigators and program planners in two ways: (i) money saved from the use of the pooling algorithm could be applied to other areas of disease prevention and/or (ii) the amount of money allocated to screening would allow more specimens to be tested for the same total cost. Pooling samples for the detection of genital *C. trachomatis* infection in urine samples is cost saving and simple to perform and could be applicable in screening programs in the United States and in population-based research worldwide.

Pooling is a technique which could be immediately used for significant cost savings in high-volume laboratories such as state labs and referral labs. Laboratories which are currently using less sensitive and specific and less costly techniques could introduce both LCR and pooling into their laboratories.

Specific populations or laboratories that might benefit from pooling include any lab in which the combination of turn-around time and volume allows at a minimum a combination of 19 pools and retests per day. With 96 specimens at a population prevalence of about 4%, pooling by six would fill up one full run (38 test unit doses) per day. The run would include, on average, 16 pools of six and 22 retests.

Laboratory managers should consider two points before using pooling. First, processed specimens from presumptive-positive pools need to be amplified and detected individually. This additional step adds a minimum of 3 hours until individual test results for specimens in presumptive-positive pools are known.

Second, laboratory managers should estimate the cost savings they expect to gain for their laboratories. This estimate is a combination of both technicians' salaries and their benefits, institutional overhead, and the prevalence of chlamydia in the populations served by the laboratory. Pooling a greater number than is recommended for certain population prevalences can cost more money than testing specimens individually.

A potential limitation of the pooling algorithm is the possibility of technician error while processed samples are pooled in the LCR run. The use of tray maps simplifies this process. Samples should be organized by skipping a space after each pooled group in the specimen rack. Thus, pooling adds no significant complexity to setting up unit doses. Additional technician error can be avoided when samples from presumptive-positive pools (detected in the previous run) are retested individually before the routine testing of the new pooled groups. Therefore, each run has a combination of samples that are retested individually and new pooled samples from the next batch of specimens.

The study laboratory has met Clinical Laboratory Improvement Act requirements for the modification of a clinical laboratory procedure from a Food and Drug Administration-approved diagnostic kit. Investigators consider performance documentation of the required study adequate for including the pooling protocol in testing clinical specimens in the study laboratory. Each laboratory that wishes to introduce pooling must meet the requirements to modify a Food and Drug Administration-approved package insert. These requirements include meeting the regulations as set forth in the Federal Register (3a).

Use of pooling processed urine samples for LCR testing of *C. trachomatis* will decrease the cost of screening, providing more evidence that screening programs can and should be implemented. Further applications of pooling include pooling urine specimens for the LCR detection of *Neisseria gonorr*

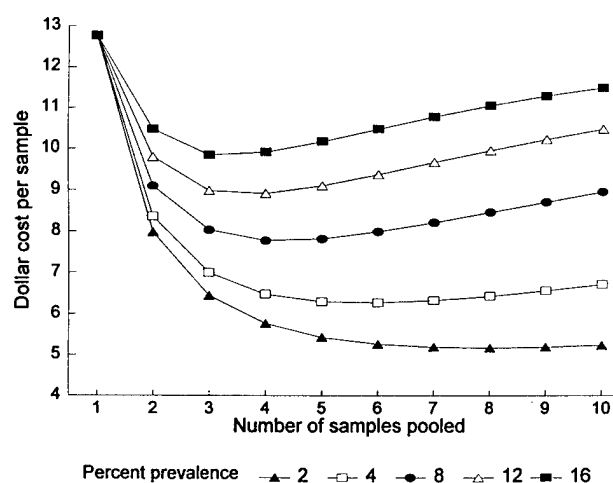


FIG. 2. Cost-saving ability of pooling processed urine specimens before the performance of the LCR test for the detection of genital *C. trachomatis* infections. The graph shows the cost per sample when the pooling algorithm was used, depending on the number of specimens pooled and taking into account various prevalences of infection in the population screened. A baseline total cost of \$12.76 per individual sample which included laboratory consumables, technician time, and LCR unit dose costs was used.

rhoeae. The cost savings of pooling urine for both *N. gonorrhoeae* and *C. trachomatis* should also be considered.

ACKNOWLEDGMENTS

We acknowledge D. Perkins for her collaboration and T. and A. Kacena for their assistance.

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CHLAMYDIA TRACHOMATIS INFECTIONS IN FEMALE MILITARY RECRUITS

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ABSTRACT

Background Asymptomatic genital *Chlamydia trachomatis* infections in women can lead to pelvic inflammatory disease, infertility, and ectopic pregnancy. To design a chlamydia-control program, we conducted a large survey of women in the U.S. military.

Methods From January 1996 through December 1997, urine samples from 13,204 new female U.S. Army recruits from 50 states were screened by ligase chain reaction for *C. trachomatis* infection. Information on potential risk factors was obtained by questionnaire. With multivariate analysis, we identified criteria for a screening program.

Results The overall prevalence of chlamydial infection was 9.2 percent, with a peak of 12.2 percent among the 17-year-old recruits. The prevalence was 15 percent or more among the recruits from five southern states. The following risk factors were independently associated with chlamydial infection: having ever had vaginal sex (odds ratio for infection, 5.9), being 25 years of age or less (odds ratio, 3.0), being black (odds ratio, 3.4), having had more than one sex partner in the previous 90 days (odds ratio, 1.4), having had a new partner in the previous 90 days (odds ratio, 1.3), having had a partner in the previous 90 days who did not always use condoms (odds ratio, 1.4), and having ever had a sexually transmitted disease (odds ratio, 1.2). A screening program for subjects 25 years of age or less (87.9 percent of our sample) would have identified 95.3 percent of the infected women.

Conclusions Among female military recruits, the prevalence of chlamydial infection is high. A control program that screens female recruits who are 25 years old or younger with urine DNA-amplification assays has the potential to reduce infection, transmission, and the sequelae of chlamydial infection. (N Engl J Med 1998;339:739-44.)

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MORE than 4 million urogenital *Chlamydia trachomatis* infections occur in the United States annually.^{1,2} They occur in young, sexually active persons from all socioeconomic groups, with prevalence ranging from 5 percent to 20 percent.^{3,4} Women, especially, bear the burden of disease, with consequences of genital infections ranging from pelvic inflammatory disease to ectopic pregnancy and infertility.^{1,5} These sequelae are associated with a large economic burden.^{6,7} Because up to 80 percent of infected women are asymptomatic and therefore do not seek

medical care, screening of young, sexually active women has been recommended.^{1,8} In the past, screening for *C. trachomatis* infections in women has been limited by the need for access to a medical clinic and a pelvic examination. However, *C. trachomatis* infections can now be detected with high sensitivity (85 to 95 percent) and specificity with DNA-amplification assays performed on urine specimens,⁹⁻¹⁴ allowing cost-effective screening of large numbers of women in nonclinic settings.¹⁵

Few studies of the prevalence of chlamydial infection in U.S. military populations have been published, and there have been no studies using DNA-amplification techniques among women not seeking health care.¹⁶⁻²⁰ Because adolescents have the highest prevalence of disease and most military recruits are young, we conducted a large prevalence study and risk-factor analysis of female recruits from throughout the United States who began basic training at Fort Jackson, South Carolina. We performed this study to determine the extent of infection, assess the feasibility of screening urine specimens for *C. trachomatis* by the ligase chain reaction, and assess which epidemiologic correlates would be useful for implementing an effective chlamydia-control program for female recruits.

METHODS

Population and Specimens

All female Army recruits who were present on Sundays between January 1996 and December 1997 at the Physical Examination Section, Reception Battalion, Fort Jackson, South Carolina, were invited to participate in this study. The study was approved by the institutional review boards of Johns Hopkins University and Fort Jackson (Eisenhower Army Medical Center, Fort Gordon, Ga.), as well as the Human Subjects Research Review Board of the U.S. Army Surgeon General. Of the 16,593 recruits approached, 13,223 (79.7 percent) volunteered to participate in the study and were given a briefing about the study as well as an educational briefing about chlamydial infections by the civilian research nurse.

All subjects signed an informed-consent form and completed a questionnaire regarding demographic information, home state,

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and sexual history. The data instrument was a two-sided scannable form (Scantron, Tustin, Calif.). To determine the similarity of the study subjects and those who chose not to participate in the study with regard to demographic characteristics and sexual history, 823 of the 3370 women who did not volunteer were invited to fill out an anonymous questionnaire. Nonvolunteers were asked to fill out a questionnaire only during the first week of each month.

Each volunteer was instructed to collect 20 to 30 ml of first-catch urine (the first part of the urine stream). A unique study number was assigned to each volunteer. All urine specimens, consent forms, and questionnaires were shipped to the Johns Hopkins University chlamydia laboratory. Urine specimens were kept at 4°C until processed, within 48 hours.

Laboratory Procedures and Treatment

Urine specimens were processed and tested by the ligase chain reaction (Abbott Laboratories, Abbott Park, Ill.) for chlamydial DNA according to the manufacturer's directions. Each week a list of infected subjects was sent to the research nurse. The infected subjects were contacted and treated at the Troop Medical Clinic at Fort Jackson by directly observed therapy with a single 1-g dose of azithromycin. The subjects were also tested for coexisting sexually transmitted diseases. The sensitivity and specificity of the ligase chain reaction in urine specimens as compared with cervical culture for chlamydia had been previously determined to be 88.6 percent and 99.7 percent, respectively, in another military population.¹⁴

Statistical Analysis

Questionnaire forms were scanned into a data base (dBASE III Plus, Borland International, Spring Valley, Calif.). The results of the ligase chain reaction, demographic information, and risk-factor information were analyzed as dichotomous variables with the chi-square test. Univariate and multivariate logistic-regression analysis for factors associated with chlamydial infection was performed with Intercooled Stata software (version 4.0, Stata, College Station, Tex.). All independent variables were entered into the model, and a two-sided P value of less than 0.05 was considered to indicate statistical significance. The 95 percent confidence interval for the prevalence value for recruits from each state was calculated with Stata software. A one-way analysis of variance was performed to assess the degree of significance of differences in prevalence between states.

RESULTS

Characteristics of the Subjects

Of 13,223 subjects presenting at the Physical Examination Section on Sundays from January 1996 through December 1997, 19 could not be evaluated because of missing data or insufficient urine. The median age of the 13,204 who could be evaluated was 21 years (range, 17 to 39); 87.9 percent (11,603) were 25 years old or younger (Table 1). Fifty-one percent of the women were white, 35.9 percent were black, and 13.1 percent were of other races. For the entire population, the prevalence of *C. trachomatis* infection according to the urine ligase chain reaction was 9.2 percent.

On the questionnaire, 93.1 percent of the subjects reported having ever had vaginal sex, 26.7 percent having had more than one sex partner in the previous 90 days, and 31.4 percent having had a new sex partner in the previous 90 days. Only 16.9 percent reported that their partners always used condoms. A history of chlamydial infection was reported by 9.1

TABLE 1. CHARACTERISTICS OF 13,204 FEMALE ARMY RECRUITS SCREENED FOR *CHLAMYDIA TRACHOMATIS*.

CHARACTERISTIC	VALUE
Age — yr	
Median	21
Range	17–39
Race — no. (%) [*]	
White	6,715 (51.0)
Black	4,733 (35.9)
Other	1,726 (13.1)
Ever had vaginal sex — no. (%) [†]	12,281 (93.1)
Sexual history in previous 90 days — no. (%)	
More than one partner [‡]	3,478 (26.7)
New partner [§]	4,076 (31.4)
Partner always used condoms [¶]	2,115 (16.9)
Previous diagnosis of sexually transmitted disease — no. (%)	
<i>Chlamydia trachomatis</i>	1,206 (9.1)
<i>Neisseria gonorrhoeae</i>	430 (3.3)
Syphilis	74 (0.6)
Trichomonas	611 (4.6)
None	11,372 (86.1)
Chlamydia-positive — no. (%)	1,219 (9.2)

^{*}Data were missing for 30 subjects.

[†]Data were missing for 9 subjects.

[‡]For 168 subjects, data were missing or subject did not know answer.

[§]For 225 subjects, data were missing or subject did not know answer.

[¶]For 684 subjects, data were missing or subject did not know answer.

percent of the subjects, gonorrhea by 3.3 percent, syphilis by 0.6 percent, and trichomonas infection by 4.6 percent. Of the volunteers who reported having had no vaginal sex, 1.4 percent (13 of 914) were chlamydia-positive, and of those who reported that their partners always used condoms, 8.4 percent (177 of 2115) were chlamydia-positive.

Of the 823 nonvolunteer recruits who filled out a questionnaire anonymously, 203 (24.7 percent) did not provide their ages and were dropped from the analysis. The mean age of the remaining nonvolunteer recruits was 21 years (range, 17 to 36); 51.3 percent were white, and 31.9 percent were black. The mean age and the racial distribution of these recruits were not significantly different from those of the volunteers. Only 66.9 percent reported having had vaginal sex, as compared with 93.1 percent of the volunteers ($P<0.001$). This group differed significantly from the volunteers in four variables, even after adjustment for whether the women reported having had vaginal sex: only 4.0 percent reported prior chlamydial infections ($P=0.013$), 18.2 percent had had a new sex partner in the previous 90 days ($P=0.002$), 20.1 percent had partners who consistently used condoms ($P<0.001$), and 90.7 percent reported no previous diagnosis of a sexually transmitted disease ($P=0.001$). Of the nonvolunteers, 17.7 percent had had more than one sex partner in the previous 90 days; the proportion of the volunteers who had had more than

one sex partner in the previous 90 days was similar after adjustment for vaginal sex ($P=0.189$).

Prevalence of Infection

The age-specific prevalence of *C. trachomatis* infection among the 13,204 volunteers is shown in Figure 1. The highest prevalence of chlamydial infection (12.2 percent) was among 17-year-olds. The prevalence declined sharply with increasing age, to below 5 percent for women over 25 years of age. For further analysis, the youngest age groups (17 to 25 years) were combined into a category called "young." The prevalence in this group was 10.0 percent (1162 of 11,603). In the older-age category (26 to 39 years), the prevalence was 3.6 percent (57 of 1601). The prevalence was 5.5 percent (369 of 6715) for whites, 14.9 percent (707 of 4733) for blacks, and 8.1 percent (143 of 1756) for other races.

Univariate Analysis

Univariate analysis identified 10 variables significantly associated with chlamydial infection: young age (17 to 25 years), black race, race other than white or black, ever having had vaginal sex, having had more than one sex partner in the previous 90 days, having had a new sex partner in the previous 90 days, having had a partner who did not always use condoms in the previous 90 days, a prior diagnosis of gonorrhea, a prior diagnosis of trichomonas, and a history of any sexually transmitted disease (Table 2). A prior diagnosis of chlamydia or syphilis was not significantly associated with being positive for chlamydial infection.

Multivariate Analysis

In the complete multivariate model, having had vaginal sex, an age of 25 years or less, black race, hav-

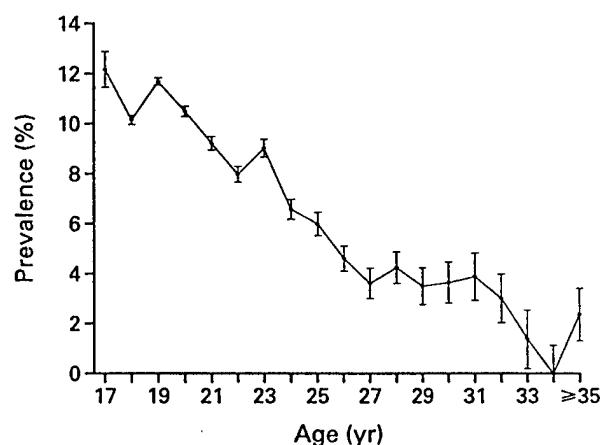


Figure 1. Mean (\pm SE) Age-Specific Prevalence of Chlamydial Infection among 13,204 Female Army Recruits, According to Ligase-Chain-Reaction Assays of Urine Specimens.

ing had more than one sex partner in the previous 90 days, having had a new sex partner in the previous 90 days, having had a partner who did not always use condoms in the previous 90 days, and a history of any sexually transmitted disease were independent predictors of chlamydial infection (Table 3).

Strategies for Selective Screening

A screening strategy involving all variables identified as independent predictors would require that 100 percent of the population be tested and would detect 100 percent of the positive subjects. In this model, the magnitude of risk associated with having had a new sex partner might vary according to race. For the purpose of a screening program, this would not alter the proportion of the population tested or the percentage of positive subjects detected with this model. Because screening on the basis of race would probably be viewed as inequitable, a strategy excluding race was examined. According to this strategy,

TABLE 2. UNIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH CHLAMYDIAL INFECTION IN FEMALE ARMY RECRUITS.*

RISK FACTOR	No. OF RECRUITS	PREVALENCE OF INFECTION		
		RISK FACTOR PRESENT	RISK FACTOR ABSENT	ODDS RATIO (95% CI)
Age ≤ 25 yr	11,603	10.0	3.6	3.0 (2.3-4.0)
Black race†	4,733	14.9	5.5	3.0 (2.7-3.5)
Other (nonwhite, nonblack) race†	1,726	8.1	5.5	1.5 (1.2-1.9)
Having ever had vaginal sex	12,281	9.8	1.4	7.5 (4.4-13.1)
Having had >1 sex partner in previous 90 days‡	3,478	13.5	7.7	1.9 (1.7-2.1)
Having had a new sex partner in previous 90 days§	4,076	12.4	7.8	1.7 (1.5-1.9)
Having had a partner who did not always use condoms in previous 90 days¶	10,405	9.8	NA	1.2 (1.0-1.4)
Condom use unknown¶	418	5.0	NA	0.6 (0.4-0.9)
Data on condom use missing¶	266	1.5	NA	0.2 (0.1-0.5)
Previous diagnosis of <i>Neisseria gonorrhoeae</i>	430	12.3	9.1	1.4 (1.0-1.9)
Previous diagnosis of trichomonas	611	11.6	9.1	1.3 (1.0-1.7)
History of any sexually transmitted disease	1,828	10.5	9.0	1.2 (1.0-1.4)

*CI denotes confidence interval, and NA not available.

†The reference group consisted of the white subjects.

‡The variable is dichotomized: the reference group consisted of the subjects who did not have >1 sex partner, who answered that they did not know, or for whom data were missing.

§The variable is dichotomized: the reference group consisted of the subjects who did not have a new sex partner, who answered that they did not know, or for whom data were missing.

¶The variable is dichotomized: the reference group consisted of the subjects who had a partner who always used condoms.

TABLE 3. MULTIVARIATE ANALYSIS OF FACTORS INDEPENDENTLY ASSOCIATED WITH CHLAMYDIAL INFECTION IN FEMALE ARMY RECRUITS.

RISK FACTOR	ODDS RATIO (95% CI)*
Age ≤ 25 yr	3.0 (2.3-4.0)
Black race†	3.4 (2.9-3.8)
Other (nonwhite, nonblack) race‡	1.7 (1.4-2.1)
Having ever had vaginal sex	5.9 (3.2-10.6)
Having had >1 sex partner in previous 90 days‡	1.4 (1.2-1.7)
Having had a new sex partner in previous 90 days§	1.3 (1.1-1.6)
Having had a partner who did not always use condoms in previous 90 days¶	1.4 (1.1-1.6)
Having ever had a sexually transmitted disease	1.2 (1.0-1.4)

*CI denotes confidence interval.

†The reference group consisted of the white subjects.

‡The reference group consisted of the subjects who did not have >1 sex partner, who answered that they did not know, or for whom data were missing.

§The reference group consisted of the subjects who did not have a new sex partner, who answered that they did not know, or for whom data were missing.

¶The reference group consisted of the subjects who had a partner who always used condoms.

recruits would be tested if they were 25 years of age or less or if they reported on a questionnaire having had more than one sex partner or a new sex partner in the previous 90 days, having had a partner who did not use condoms in the previous 90 days, or having a history of sexually transmitted disease. Screening according to these criteria would still require testing 100 percent of the population. If a questionnaire could be avoided and young age (25 years or less) alone was the screening criterion, 87.9 percent (11,603 of 13,204) of the population would need to be tested and 95.3 percent (1162 of 1219) of the positive subjects would be identified.

Geographic Variation in Prevalence

There was considerable variation in the prevalence of chlamydial infection according to the state of origin of the recruits ($F < 0.001$ by one-way analysis of variance). The prevalence was more than 15 percent for recruits from South Carolina, Georgia, Alabama, Louisiana, and Mississippi. For New Jersey, North Carolina, Kentucky, Texas, Oklahoma, and Arkansas, the prevalence was 10 to 15 percent, and for 17 other states and Puerto Rico, it was 5 to 10 percent. For five states (Washington, Oregon, Minnesota, Arizona, and Massachusetts), the prevalence was less than 5 percent. Fewer than 100 recruits were tested from each of 17 states, 3 territories, and the District of Columbia, and prevalence figures from these areas were therefore not included in the analysis. The prevalence for the five states with the highest prevalence and the five states with the lowest prevalence differed significantly, since the 95 percent confidence intervals for prevalence did not overlap.

DISCUSSION

Although the diagnosis and treatment of sexually transmitted diseases has always presented a challenge, there has been no routine screening of recruits for chlamydial infections at entry into the U.S. Army.²¹ Because most chlamydial infections are asymptomatic in women and because the sequelae of disease present a severe and costly burden, screening women at entry into the Army is an appropriate way to identify infections early and to explore opportunities for a control program.^{1,6,7,22}

Civilian chlamydia-control programs have sought to identify criteria for selective screening.²³⁻²⁷ Most of these control programs have used diagnostic assays that require pelvic examinations and cervical specimens.²⁵ However, it has recently been shown that testing urine specimens by DNA-amplification techniques is cost effective for screening large numbers of persons in different settings.^{15,28} We used this new technique to determine the prevalence of chlamydia and to identify screening criteria for a program to control chlamydia in the military.^{10,12,14} Collection of urine specimens in this study was highly acceptable and easily implemented.

Using the ligase chain reaction with urine samples, we found a high prevalence of *C. trachomatis* infection (9.2 percent). This prevalence was higher than that observed in family-planning clinics²⁸ but not as high as that reported in some adolescent health clinics.^{29,30} Our data agree with those from previous studies of chlamydial infections in Army women, in which prevalence rates ranged from 8.2 percent to 9.8 percent.^{17,18} In one large, community-based screening study, the overall prevalence of chlamydia in young women was 8.6 percent, as detected by the urine ligase chain reaction, a prevalence similar to that found in our study.³¹ Because our population was not clinic-based and was not made up of women seeking health care, the finding of such a high prevalence in these women warrants the institution of a control program for the routine identification and treatment of chlamydial infections in order to prevent sequelae and transmission to sex partners.⁸

The study population consisted of a young, sexually active group of female recruits with sexual risk factors known to be associated with chlamydial infection.²⁵ Although 9.1 percent of the subjects reported having had chlamydial infection in the past, this factor was not associated with the risk of current infection. The highest prevalence was observed among 17-year-olds. This prevalence is similar to comparable age-specific rates in other studies, confirming that young age is associated with chlamydial infection.^{28,31} In our study, young age was associated with being chlamydia-positive in both univariate and multivariate analyses (odds ratio, 3.0). In order to include more positive subjects, we used an age cutoff of 25 years, which allowed the detection of 95.3 per-

cent of the chlamydial infections. Other studies have supported age-based screening for chlamydia.^{27,31,32}

Thus, for this group of female recruits coming from a civilian background, who were tested within three days of starting basic training, young age alone can be recommended as a single indicator of who should be tested for chlamydial infection. Other models considered in this study offered high sensitivity, but the models were more complex and required valid sexual-risk histories. We documented 13 chlamydial infections (prevalence, 1.4 percent) among 914 recruits who denied being sexually active, as well as chlamydial infections in 8.4 percent of those who reported that their partners consistently used condoms. These figures indicate that self-reported sexual-risk histories are not always valid.³³ The lower prevalence of chlamydial infection among recruits for whom the data on condom use were missing, or who indicated on their questionnaires that they did not know whether their partners always used condoms, may be due to lack of sexual activity, because 58.6 percent of the 684 recruits in these categories reported that they had never had vaginal sex. There is a fixed laboratory budget available for population screening in the Army. Young age is the simplest, least expensive, and most easily documented risk factor on which to base a recommendation for a screening program, as well as being highly sensitive. Alternatively, since the use of age as a selective screening criterion would have missed 4.7 percent of the infections, universal screening might be more cost effective from a societal perspective, and future studies of cost effectiveness are warranted.²⁸

This was one of the largest programs for screening young, sexually active subjects that was not clinic-based and whose results were derived from urine DNA-amplification assays. The geographic variation in prevalence was striking. From more than 15 percent in the five states with the highest prevalence to less than 5 percent in the five states with the lowest, these differences may reflect the levels of disease burden in certain states. These regional variations also appear to reflect regional differences in chlamydial disease, as reported by the Centers for Disease Control and Prevention.^{34,35} For example, the prevalence in North Carolina reportedly varied from 10 percent to 17 percent.³⁵ The prevalence is lower in regions such as Wisconsin and Washington State, where clinic-based chlamydia-control programs are in place and where declining rates of prevalence of chlamydia have been reported.^{26,27,32,36} In our study, the prevalence was 11.3 percent for North Carolina and 3.8 percent for Washington State. Our data imply that chlamydial infection remains common in young women across the United States. With a volunteer rate of 80 percent among women who were approached and representation from 50 states and 4 territories, our study had a wide geographic sampling.

One limitation of our study is that it is not known whether the prevalence of risk factors for chlamydial infection differs between young women who decide to join the military and those who do not. However, the demographic and sexual risk-factor characteristics of our subjects appear to be similar to those of other regional and clinic-based populations,²⁵ as well as those from a large, community-based study.³¹ An additional limitation is that the nonvolunteers in our study differed from the volunteers with regard to sexual risk factors for chlamydia. However, the nonvolunteers represented a group who were mostly sexually active, who had had new sex partners in the previous 90 days, and whose partners did not use condoms. Thus, their risk of chlamydial infection may have been as high as that of the subjects in our study.

Although amplified-DNA tests are more expensive than traditional nonculture tests, the savings associated with not having to have a clinician collect specimens from a pelvic examination and the advantages of being able to use urine as a diagnostic specimen may outweigh the extra cost of the test.¹⁵ In addition, it has been demonstrated that amplified-DNA testing of urine specimens is cost effective, and treating chlamydial infections prevents serious complications such as pelvic inflammatory disease, ectopic pregnancy, and infertility.^{15,28,37}

In conclusion, our study indicates that with the limited funding available at present, young age (25 years or less) would be the best criterion on which to base a screening program using amplified-DNA testing of urine for female Army recruits and perhaps for other young women. Institution of such a control program has the potential to reduce drastically the burden of chlamydial disease in the U.S. Army and to prevent morbidity due to these infections.³⁷

Supported by a grant (DAMD17-95-1-5064) from the Department of the Army.

The opinions expressed in this article are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense.

We are indebted to Dr. Daniel Scharfstein, Mary Sheppard, Dr. Guillermo Madico, and Dr. Anne Rompalo for statistical assistance; to Kathryn Kacena for data entry; to Diane Perkins, Sandra Leister, Dien Pham, and Graciela Jaschek for performance of the laboratory tests; to Lt. Col. Cynthia Dodd, Lt. Col. (ret.) Delois Daniels, and Kimberly Riley for treatment of infected women and processing of specimens; and to Patricia Buist for assistance in the preparation of the manuscript.

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9.2 Abstracts presented at conferences during the project's third year

9.2.1 International Symposium of Sexually Transmitted Disease Research, Seville 1997.

9.2.1.1 Howell, M.R., McKee, K., Ellis, D., et al. Cost-effectiveness of screening vs mass therapy for *C. trachomatis* in female army recruits

9.2.1.2 Stability of *C. trachomatis* and *N. gonorrhoeae* in urine at room temperature for LCR and PCR tests.

9.2.2 Conference on the Macrolides, Azalides, Streptogramins, and Ketolides, Barcelona, 1998: Howell, M.R., Quinn, T.C., Hendrix, R., et al. Single-dose azithromycin for mass therapy to control *Chlamydia trachomatis* in female Army recruits: a cost-effectiveness model.

9.2.3 International Conference on Emerging Infectious Diseases, Atlanta 1998: Gaydos, C.A., Howell, M.R., Clark, K.L., et al. A silent epidemic of *Chlamydia trachomatis* genital disease in female U.S. Army recruits.

P511 CHLAMYDIA TRACHOMATIS INFECTIONS IN SYMPTOMATIC AND ASYMPTOMATIC COUPLES

Conclusion: In symptomatic as well as in asymptomatic women significantly more chlamydial infections could be detected when also testing their male partners. Testing of one male (urine) and one female sample (urine or cervical swab) by LCR appears to be more cost-efficient than testing several female samples.

P512 COST-EFFECTIVENESS OF URINE-BASED S
TRACHOMATIS WITH LIGASE CHAIN REACTI

Conclusions: For asymptomatic young men outside ST, LCR is cost-effective at CT prevalence $\geq 6\%$. Although LCR detection, its use can reduce the cost of a comprehensive CT prevalence is $\geq 10\%$.

Conclusion: Mass therapy in a well defined cohort of young women with a high prevalence of CT would prevent sequelae, would likely decrease the number of discharges for medical reasons in the first 6 mos. of service, and would save overall costs.

Results: LCR (+) results were 528 (2.8%); distributed: 3.4% men 5/205 (2.4%); in group B 2/79 (2.5%). The prevalence according to age in group A: women 15–20 years old 1.7%, 21–25 years old 1.1%. For women in group B LCR (+): LET (-) 5/5 LCR (+), for men LET (-) 1/4 LCR (+). The sensitivity and specificity of LET was 100% and 40% respectively.

Conclusions: This is the first prevalence study for C.T. in Uruguay. There was no available non-invasive test to study with low prevalence of C.T. infection. We found LCR we found infection in women between 15–20 years old (4%), as in this age should not be used as screening in this kind of population. LCR are cost effective even in asymptomatic women in our country.

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In the Netherlands, the municipal policy of toleration and de-criminalisation towards prostitution has led to a debate over its regulation and legislation. Organised prostitution in the Netherlands is forbidden, while in practise brothels are allowed to operate with varying amounts of freedom. This semi-legal status gives brothel owners the freedom to do business, without enabling local authorities to impose punishment for operating infractions. In anticipation of a change in the national law, the Municipality of Amsterdam developed their own new regulation. Since Jan 96: i) it is illegal for women from outside the EC to work as prostitute and ii) brothel owners can get a license if they fulfill certain conditions (hygiene, fire safe, no employment of illegal women).

In Amsterdam, uptill 1996, prostitutes working in windows were mainly Latin American women. As research revealed that they and their clients were at increased risk for both STD and HIV, public health nurses (together with cultural mediators) frequently visited these women in order to give information on safe sex, HIV/STD, birth control and tuberculosis and to inform them about our STD clinic. In 1995 first contacts were made with 996 prostitutes, including 569 Latin American prostitutes, 73 African, 134 Eastern-European and 58 Asian women. In 1996 the number of contacts decreased considerably (n=651), despite a similar frequency of fieldwork. Where did the women disappear to? The consequences of this new regulation will be discussed with regard to accessibility of the women and the segregation of prostitution networks, including the increased risk for STD/HIV transmission.

0174 THE USE OF LIGASE CHAIN REACTION (LCR) IN THE DIAGNOSIS OF GONOCOCCAL AND CHLAMYDIAL EPIDIDYMITIS.

Fehler HG, Radebe F, Dangor Y, Grobbelaar T, Ballard RC. National Reference Centre for STDs, SA Institute for Medical Research, and University of the Witwatersrand, Johannesburg, South Africa.

Objective: A study was conducted to determine the value of the ligase chain reaction (LCR) technique as a diagnostic tool in cases of epididymitis.

Methods: Ninety five consecutive cases of acute epididymitis among migrant mineworkers attending an STD clinic at the Leslie Williams' Memorial Hospital, Carletonville, were included in the study. In each case, endocervical swabs were collected for gonococcal and chlamydial cultures. A first catch urine sample was collected for LCR for both *Neisseria gonorrhoeae* (GC-LCR) and *Chlamydia trachomatis* (CT-LCR). In addition, blood specimens were tested for syphilis, chlamydial and HIV antibodies.

Results: Overall, either by culture or LCR, 43 patients (45%) had evidence of gonococcal infection, while 25 patients (26%) had proven chlamydial infection. A further 23 cases (24%) had high levels of antichlamydial antibody of $\geq 1:64$ using the indirect microimmunofluorescence test (micro-IF). Of the 25 cases with proven chlamydial infection, 13 cases also had concomitant gonococcal infections (52%). Among the cases with gonococcal infection, 84% were detected by GC-LCR, while only 67% were cultured. Similarly, using CT-LCR, 88% of chlamydial infections were detected, while only 40% were culture-positive. In 25 cases (26%) no evidence of infection with either organism was obtained. Reactive syphilis serology was found in 12% of cases and 39% were HIV seropositive.

Conclusions: LCR proved to be more sensitive than culture for the diagnosis of epididymitis. Chlamydial micro-IF serology proved a useful adjunct to LCR for the diagnosis of chlamydial infection. In approximately a quarter of all cases no aetiology could be determined.

0176 CHLAMYDIAL DIAGNOSIS WITH THE LCX, COBAS AMPLICOR, AND TRANSCRIPTION MEDIATED AMPLIFICATION USING VULVAL SAMPLES

Stary A, Hartmann B, Schuh E, Kerschbaumer M. Outpatients' Centre for Infectious Venero-Dermatological Diseases, Vienna, Austria.

Objective: To evaluate whether vulval smears may serve as an alternative non-invasive specimen type for chlamydial diagnosis, the performance of all commercially available amplification tests (LCx *Chlamydia trachomatis* assay, COBAS Amplicor, Gen-Probe Transcription-Mediated Amplification) with vulval specimens as well as with urine, and cervical samples was compared with culture on endocervical and vulval samples.

Methods: The study was performed in female patients attending the Outpatients' Centre mainly because of a suspected genital tract infection, partner control, contact tracing, and health check up. In addition to first void urine (FVU), samples were obtained from the vulval region and endocervix and tested by culture and amplification tests.

Results: The sensitivity (Table) and specificity for all sampling sites and amplification methods was high, calculated on infected women as the goldstandard established by positive culture or 2 different test procedures.

Test Method	Vulva	FVU	Cervix
LCR (77/875)	89.6% (69)	90.9% (70)	92.2% (71)
TMA (25/308)	92.0% (23)	76.0% (19)	88.0% (22)
PCR (25/398)	88.0% (22)	80.0% (21)	88.0% (22)
Culture (77/875)	14.3% (11)	n.d.	59.7% (46)

Conclusion: The results demonstrate that in contrast to culture, all amplification methods performed with a high sensitivity with vulval smears (89.8%), comparable with urine (86.6%) and cervical specimens (90.6%). The data indicate that vulval smears can be used as alternative noninvasive specimens useful for chlamydial screening programs.

Van Voorhis, WC, Patel B, Cosgrove S, University of Washington Medical School, WA USA.

Objective: Ct has been detected by a variety of semi-quantitative techniques, however a quantitative detection system is needed to follow the levels of Ct in tissues after therapy. A quantitative competitive PCR (QC-PCR) technique was developed for quantitating Ct during infection.

Methods: The PCR uses the plasmid found in Ct as a template, yielding a 442 bp band from Ct DNA. A competitor template was constructed in a plasmid (p337) containing the PCR product with an internal deletion of 105 bp, yielding a 337 bp PCR product. DNA was purified from Ct scrovar E. Autologous salpingeal tissue grafts were established in subcutaneous pockets of *Macaca nemestrina* (pigtail macaque) and either inoculated with 10^3 IFU of Ct Scrovar E or sham-inoculated. DNA was extracted from salpingeal tissues harvested at day 7 post-inoculation.

Results: Serial dilutions of Ct DNA were mixed with 0.01 fg of p337 competitor plasmid (~300 copies) and subjected to PCR. At 10 to 0.3 pg of Ct DNA, the Ct 442 bp band predominated in the PCR products. At 0.03 to 0.001 pg of Ct DNA, the competitor 337 bp band predominated. The equivalence point, where the intensity of the competitor band equaled that of the native template band, was found at 0.1 pg. Because each Ct organism has ~1fg of DNA, the DNA of approximately 100 Ct appeared to equal that of ~300 p337 plasmids. This makes sense, because there are between 2 to 7 copies of the target template (chlamydia plasmid) in each Ct. PCR using DNA from the Ct-inoculated salpingeal tissues, but not from sham-inoculated tissue, yielded the expected 442 bp product. Serial dilutions of Ct-inoculated DNA gave equivalence with 0.01 fg of p337 using 1/100 of the total DNA extracted. Since 0.01 fg p337 was equivalent to 100 Ct organisms, this suggests that 10,000 Ct were present in each Ct-inoculated salpingeal pocket.

Conclusion: QC-PCR is an effective method for measuring the quantity of Ct in infected tissues.

0175 URINE LIGASE CHAIN REACTION FOR THE DIAGNOSIS OF LYMPHOGRANULOMA VENEREUM IN SOUTHERN AFRICA.

Radebe F, Fehler HG, Ye Hrun, Tshabalala V, Ballard RC. National Reference Centre for STDs, SA Institute for Medical Research, and University of the Witwatersrand, Johannesburg, South Africa.

Objective: To compare ligase chain reaction (LCR) assay for *Chlamydia trachomatis* with standard culture and serological techniques for the diagnosis of lymphogranuloma venereum (LGV).

Methods: One hundred and ninety seven consecutive men with a clinical presentation consistent with LGV were enrolled in the study. In each case swabs were taken from the urethra and, if present, genital ulcerations for isolation of *C. trachomatis*. A serum specimen was obtained for micro-IF serology. A first catch urine specimen was collected for detection of *C. trachomatis* DNA using the LCR technique. Additional tests were performed to exclude infection with *H. ducreyi*, *T. pallidum* and HSV.

Results: Of 197 patients entered in the study, no laboratory evidence of LGV was found in 38 cases (19%). There was 148 evaluable cases, of which 44 (30%) were culture-positive for *C. trachomatis*, while urine LCR was positive in 63 cases (43%). Elevated antichlamydial antibody levels ($\geq 1:128$) were detected in 130 cases (88%). A combination of culture and serology resulted in a diagnosis of LGV in 135 cases (91%). When urine LCR and serology were used for diagnosis, 143 cases were detected (97%). LCR proved significantly more sensitive than culture when no ulcers were present. However, no significant difference between techniques could be detected when primary lesions were present.

Conclusions: Urine LCR proved to be a convenient, non invasive adjunct to micro-IF serology for the diagnosis of LGV in an area where the disease is commonly encountered.

0177 STABILITY OF *C. TRACHOMATIS* AND *N. GONORRHOEA* IN URINE AT ROOM TEMPERATURE FOR LCR AND PCR TESTS.

Gaydos CA¹, Welsh L¹, Kralian S¹, Perkins D¹, Schmidt K¹, Chou J¹, Quinn TC^{1,2}. ¹The Johns Hopkins Univ., Baltimore, MD; ²NIAID, NIH, Bethesda, MD

Objective: To determine the stability of concentrations of *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) in urine at room temperature (25° C) for testing by LCR and PCR.

Methods: Aliquots of urine concentrations of CT from 100 to 1 IFU/ml and of NG from 10^4 to 1 CFU/ml were sequentially processed and tested by LCR and PCR at times 0 hr, 24 hr, 48 hr, 72 hr, 7 days, and 14 days.

Results: CT/LCR: All concentrations of CT from 100 to 1 IFU were stable in urine, giving positive results, until 14 days at 25°. CT/PCR: At 0 hr, concentrations from 100 to 5 IFU were positive. One IFU was negative. At 24 hr, all samples were pos. At 48 and 72 hr, concentrations from 100 to 5 IFU were positive. At 7 and 14 d, all concentrations from 100 to 1 IFU were pos. NG/LCR: At 0, 24, 48, and 72 hr, concentrations from 10^4 to 50 CFU were pos. At 7 d, only 10^4 & 10^5 were pos. At 14 d, only 10^5 was pos. NG/PCR: At 0 and 24 hr, only 10^4 and 10^5 CFU were pos. At 48 and 72 hr, only a concentrations of 10^4 CFU was positive. At 7 and 14 d, all samples were negative.

Conclusions: 1-5 IFU/ml CT appear to be very stable in urine specimens at 25° C by LCR and PCR. NG is more labile, requiring 50 CFU for detection by LCR at 24-72 hr and 10^4 - 10^5 CFU for detection by PCR at 24-72 hr.

or chlamydial cervicitis, and symptoms, and and antibody detection *Chlamydia trachomatis*. Patients were days 10 ± 2 and 21 ± 2 . until the end of the study or, as recorded at each visit. 2 (32%) had gonococcal pathogen was detected in but were included in the

10 *N. gonorrhoeae*, two and 14 (44%) improved; 30 ts had positive antibody ents were cured and three positive for *Chlamydia*; the were experienced by 7/38 and probably related to the

ccal cervicitis using a 1-g e (cure + improvement) in

the Treatment of Male cal Urethritis

5² sity of Antwerp, Antwerp,

5 g for the treatment of post-gonococcal urethritis patients were assigned to ment samples were taken and *Neisseria gonorrhoeae* % of evaluable patients in ased as microbiologically -susceptible strains of *N.* 14-21), chlamydial post- and 8/31 (25.8%) of the ively ($p=0.041$). There was between the two groups: transient nausea, which study results show that not ve as 0.5 g ciprofloxacin in er caused by penicillin- t that it is also significantly itis.

The most appropriate antibiotic treatment not c the pharmacokinetic and pharmacodynamic prop antibiotic with acceptable activity against aty transmitted diseases. Due to its pharmacokinetics genitourinary fluids and inside the cell. This leve bacterias. In this study Roxithromycin was administ of treatment.

A multicentre descriptive study was done. It involved 274 patients, both male (71 - 21.91%) and female (203 - 74.09%). Average age was of 34 years (minimum 15 years - maximum 83 years). This 274 patients reported 1005 symptoms and signs with an average of 3.66 symptoms or signs per patient. 221 patients (80.66%) were evaluated as total recovery of the symptoms and 53 patients (19.34%) persisted with at least one clinical manifestation. It is important to mention that in 5 of this 53 patients new symptoms appeared in the second visit; this symptoms were not reported in the first medical consultation.

Adverse effects were observed in 20 patients (7.3%) but only in 3 cases (1.09%) the medication was suspended.

This evaluation shows that patients with non gonococcal genitourinary infections present a good response to therapy with Roxithromycin. Besides, side effects are very low with this therapy. Adverse effects are considered not serious and Roxithromycin rarely has to be discontinued.

8.09

Single-dose Azithromycin (AZ) for Mass Therapy to Control *Chlamydia trachomatis* (CT) in Female Army Recruits: a cost-effectiveness model

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Objective: There is a high prevalence of *C. trachomatis* (CT) in female U.S. Army recruits, which may cause serious morbidity due to PID. We evaluated the cost-effectiveness of treating all recruits with AZ to prevent chlamydial sequelae compared to testing all recruits and treating only those with positive CT tests.

Methods: In a decision analysis, we modeled mass treatment and universal testing to determine the incremental cost-effectiveness of these two strategies. From a military perspective, we calculated the total program costs, the projected costs of lost training, the projected costs of sequelae due to CT, the projected level of prevented disease (PID, chronic pelvic pain, and ectopic pregnancy) and the projected number of military discharges for medical conditions. 9,192 recruits presenting for basic training at Ft Jackson, SC 1996-97 were tested by urine LCR to determine CT prevalence. Results were extrapolated to a hypothetical population of 10,000 recruits per annual cohort.

Results: The recruit sample had a CT prevalence of 9.0%. 218 cases of PID would be expected in the absence of a control program. Mass therapy would prevent 187 cases, 21 more than universal testing. Mass therapy saved \$39,500 over universal testing for a 5-yr follow-up period and \$357,300 over no program. At CT prevalence > 6.2% and fewer than 14.1 clinic visits per 100 individuals for moderate to severe side-effects, mass therapy provided a cost-savings over universal testing. Both strategies provided cost-savings over no program at CT prevalences over 3%.

Conclusion: Mass therapy would prevent sequelae, would likely decrease the number of military discharges for medical reasons in the first 6 mo. of service, and would save overall costs relative to universal testing. Mass therapy could be considered as a first line of control for chlamydia in a well-defined cohort of young women with a high prevalence of CT.



dom use was noted in persons who received more recent positive test results than among persons with positive test results from earlier years ($p < 0.001$), though this trend was not noted in heterosexual men. The proportion of persons who injected drugs [IDU] decreased with more recent years of first positive HIV test [$p = 0.01$], especially among women. There were slight decreases in the proportion of persons who traded sex for money or drugs, and who used cocaine, with more recent years of first positive HIV test, but the proportion using crack increased. **Conclusions:** High-risk practices, such as sex without condoms and IDU, have decreased among MSM and women. Heterosexuals are emerging as a more prominent group for HIV infection.

P-12.3 The Structure of a Newly Identified Genetic Subtype in the HIV-1 Epidemic in Africa. J.K. CARR, F.E. MCCUTCHAN, M. EMERSON, D. BIRX. Henry M. Jackson Foundation for the Advancement of Military Medicine, Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, MD.

Objective: Genetic analysis of HIV-1 genomes has led to the description of at least 10 different genetic subtypes, each with its own geographic or demographic distribution. Although subtype A is one of the most common genetic subtypes of HIV-1 in Africa, only three full-length genomes of this subtype have been sequenced: two from Uganda and one from Nigeria. **Methods:** Two isolates, DJ263 and DJ264, characterized as subtype A by gag and env sequencing, have been cloned and sequenced in full for the first time. The entire genome was sequenced except for a 73 nt region in the LTR. These subtype 'A' viruses were from Djibouti, on the coast of East Africa. Phylogenetic analysis of the full-length genome was performed with these new isolates. **Results:** The Djibouti viruses were found to form a unique cluster in the full-length analysis, grouping with previously sequenced isolate, IbNG, from Nigeria. They were distinct from subtype A and similar to each other from the 5' to the 3' end. Detailed analysis of the subtype structure of these isolates in comparison with reference sequences of the main HIV-1 subtypes, revealed them to be complex recombinants between subtypes A, G and an original "IbNG" parental strain. Both Djibouti isolates and the Nigerian isolate had the same complex subtype structure. **Conclusions:** Full-length genomes from one of the common subtypes in Africa will be fully characterized in this report. Although related to subtype A in both gag and env, the isolates were found to be a homogeneous viral subtype of their own, with a complex subtype structure recombinant between two known subtypes, subtypes A and G, and one unknown subtype, the putative parental subtype. It is recommended that this viral subtype be given the new subtype designation of "IbNG*". Like the subtype E* viruses, which are E/A recombinants, this subtype has a segment from an original parental virus, a virus which has never been found in its 'pure', or non-recombinant, state.

Board
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P-12.4 A Silent Epidemic of *Chlamydia trachomatis* Genital Disease in Female U.S. Army Recruits. CA GAYDOS, MR HOWELL, KL CLARK, KT MCKEE, RM HENDRICKS, D ELLIS, TC QUINN, JC GAYDOS. Johns Hopkins Univ., Baltimore, MD, Walter Reed Army Inst Res, Washington, DC, Ft. Bragg, NC, Ft Jackson, SC, NIAID, NIH, Bethesda, MD, HM Jackson Fdn, Rockville, MD.

Background: *C. trachomatis* (CT) infections in the U.S. exceed 4 million cases annually, occur mostly in the young and lead to costly sequelae (PID, ectopic pregnancy, and infertility). Most (>80%) infections are asymptomatic in women, leading to epidemic rates in sexually active youth. Our hypothesis was that a large epidemic of treatable CT genital infections, related to the ongoing civilian epidemic, was occurring in female recruits. **Methods:** Ligase Chain Reaction (LCR) of urine speci-

mens was used to determine the prevalence of CT infections in female military recruits (N = 9,192) at induction. Demographics and sexual risk data were collected. **Results:** Overall prevalence was 9.0%. By questionnaire, 93.6% reported vaginal sex, 26% had >1 sex partner and 30.5% had a new partner. Only 15.5% always used condoms. By age, prevalences were: 11.0% (17-20 yr), 8.0% (21-25 yr), 3.1% (26-30 yr), 1.9% (31-35 yr). By home state, 4 states had a prevalence >15%, 8 states 10-15%, and 12 states 5-10%. By multivariate analysis, significant variables associated with CT positivity were vaginal sex (OR 4.1), young (17-25 yr) age (OR 3.4), African American (OR 2.7), > 1 partner (OR 1.4), and a new partner (OR 1.5). A model consisting of LCR screening young female recruits (17-25 yr) would test 87.2% of the recruit population and identify 95.8% of CT positives. **Conclusions:** A high prevalence of CT infection exists in female army recruits, especially those of young age. Differences by state may be representative of national, geographically diverse prevalences. Detection of infection by LCR of urine permitted a large, efficient screening program. Data collected are being used to develop a cost-effective treatment program.

P-12.5 Epidemiology of Shigellosis in San Francisco during the HIV Era. J. T. BAER, D. J. VUGIA, A. L. REINGOLD, T. ARAGON, F. J. ANGULO, and W. Z. BRADFORD, for the CA Emerging Infections Program. CA Emerging Infections Program, CA Department of Health Services, UC-Berkeley School of Public Health, Berkeley, CA; Centers for Disease Control and Prevention (CDC), Atlanta, GA; San Francisco Department of Public Health, UC-San Francisco.

Objective: The impact of HIV infection on the epidemiology of Shigellosis and its potential role as a risk factor have not been well described. To better understand this relationship and to assess the role of gay sex, a recognized risk factor for Shigellosis, we conducted an investigation of cases of Shigellosis in San Francisco, a city with a high prevalence of both HIV infection and men engaging in gay sex. **Methods:** As part of CDC's Emerging Infection Program, active surveillance for infections caused by *Shigella* species was conducted in SF during 1996. All available medical records were reviewed using a standardized data collection instrument and data previously collected by the SF Department of Public Health (DPH) during routine interviews of cases were obtained. The estimated prevalence of HIV infection and men engaging in gay sex was obtained from the SF DPH. **Results:** A total of 228 culture-confirmed cases were identified, including 142 and 73 caused by *S. sonnei* and *S. flexneri*, respectively. The incidence rate in cases per 100,000 population was 31.5, compared with an active surveillance rate of 10.9 in neighboring Alameda County (AC) and a reported rate of 7.3 in the U.S.. The incidence rate in the 25-44 year age group was 46.0 cases per 100,000 population, compared with 6.8 cases per 100,000 population in AC, and the rate in HIV-infected persons was 442 cases per 100,000 population. Adult cases (age > 17 years) comprised 80% (181/228) of the total, and had the following characteristics: male gender (75%), white race (70%), gay male (65%), HIV-infected (52%), and sexually active in the 10 days prior to interview (69%). Fifty-one percent (96/190) of infections occurred in gay, adult men. When compared to the non-gay and non-HIV infected population, the incidence rate ratios for the gay and non-HIV-infected, the non-gay and HIV-infected, and the gay and HIV-infected populations were 4.8 (95% Confidence Interval (CI) 2.7-8.0), 34.0 (95% CI 14.2-70.1), and 35.0 (95% CI 24.6-49.5), respectively.

Conclusions: These population-based data demonstrate high overall rates of Shigellosis and dramatically elevated rates in HIV-infected persons. The high prevalence of HIV infection and men engaging in gay sex are likely important determinants of the Shigellosis rates in SF. The relative contribution of behavioral factors as compared to compromised host immunity in HIV-infected individuals warrants future investigation.

9.3 Abstracts submitted during the project's third year for conferences

9.3.1 Howell, M.R., Gaydos, J.C., KcKee, K., et al. North-south-east-west: geographic, race, and age correlates in non-health care seeking U.S. female Army recruits. National STD Prevention Conference, Dallas, 1998.

9.3.2 Cecil, J.A., Howell, M.R., Gaydos, J.C., McKee, K.T. Ellis, D., Hendrix, R.M., Quinn, T.C., and Gaydos, C.A. Prevalence and risk factors of *C. trachomatis* infection in male military recruits using urine ligase chain reaction (LCR). Infectious Diseases Society of America. Denver, CO, 1999.

9.3.3 Cecil, J.A., Howell, M.R., Gaydos, J.C., McKee, K.T. Hendrix, R.M., Quinn, T.C., and Gaydos, C.A. Prevalence and risk factors of *C. trachomatis* infection in male military recruits: multivariate analysis. Prevention 99. Washington, D.C., 1999.

North-South-East-West: Geographic, Race and Age Correlates in Non-Health Care Seeking U.S. Female Army Recruits

MR Howell¹, JC Gaydos^{2,3}, KT McKee⁴, K Clark³, TC Quinn^{1,5}, CA Gaydos¹
¹ Johns Hopkins University, Baltimore, MD; ² HM Jackson Foundation, Rockville, MD; ³ Walter Reed Institute of Research, Washington, DC; ⁴ Fort Bragg, NC; ⁵ NIAID, NIH, Bethesda, MD.

Background/Rationale: Regional variations in *C. trachomatis* prevalence have been documented in clinic populations. Similar regional variations in non-health care seeking women have not been examined to determine the influence of confounding variables such as demographics. It is necessary to understand differences in populations from non-traditional settings from a diverse geographic perspective.

Objectives: To describe and assess the significance of regional variations and determine if these differences remain when controlled for race and age in a non-health care seeking military population.

Methods: From 1/96 through 12/97, we tested 13,204 female recruits by ligase chain reaction of urine for *C. trachomatis* infection upon entry to the U.S. Army. The impact of regional variations (CDC reporting areas: northeast, south, midwest, west and territories) on chlamydia prevalence among these women was analyzed by ANOVA. The effect of intervening factors (self-reported race and age) was assessed by logistic regression.

Results: An overall prevalence of 9.2% (1,219/13,204) was observed. Home state records were available for 13,152. Women coming from the northeast had a chlamydia prevalence of 6.9% (146/2,103); the south 11.9% (760/6,381); the midwest 7.1% (167/2,351); the west 5.6% (117/2,100); and the territories 10.0% (21/211), ($F < 0.001$). Region, African American race and young age (age ≤ 25) were all independent predictors of infection. Women coming from the southern states were more likely (O.R. 1.5, 95% CI: 1.3- 1.7) to be positive for chlamydia than women coming from the other four regions. Conversely women in the west were less likely (O.R. 1.4, 95% CI: 1.1-1.7) to have a positive test than other women.

Conclusions: In non-health care seeking U.S. Army recruits regional disparities in chlamydia prevalence exist. Other national studies in non-health care seeking populations are necessary.

Learning Objectives:

1. Using the results of this analysis participants will be able to describe the regional variations in a non-health care seeking population.
2. Using the results of this analysis participants will be able to describe the association between race, age, region and infection with *C. trachomatis*.



Infectious Diseases Society of America

36th Annual Meeting

Deadline: August 5, 1998

A font of 10 point or larger is acceptable.

Abstract Form

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Prevalence and Risk Factors of *C. trachomatis* Infection in Male Military Recruits Using Urine Ligase Chain Reaction (LCR)

JA CECIL^{1*}, R HOWELL¹, JC GAYDOS², KT MCKEE³, D ELLIS⁴, RM HENDRIX⁴, TC QUINN^{1,5}, CA GAYDOS¹. Johns Hopkins University¹; HM Jackson Foundation, Rockville, MD²; Ft. Bragg, NC³; Ft. Jackson, SC⁴; NIH, NIAID, Bethesda, MD⁵.

Chlamydia trachomatis urogenital infections in women are associated with significant morbidity, including PID, ectopic pregnancy and infertility. In a study of female Army recruits using urine ligase chain reaction (LCR, Abbott Laboratories, Abbott Park, IL), *C. trachomatis* was identified in 9.3% of women screened. The effectiveness of efforts to limit chlamydial infections in women may depend on the prevention of re-infection through simultaneous screening and treatment of men. We tested male recruits for *C. trachomatis* using urine LCR to determine prevalence and to assess potential screening criteria for *C. trachomatis* infection in men. Each recruit who volunteered to participate provided a urine sample and answered a questionnaire. Among 1,203 men screened, the prevalence of infection was 4.9%. The mean age was 19.8 (+2.61) years, 61.6% were Caucasian, 33.8% had more than one sex partner, and 36.7% had a new sex partner in the last 90 days. Only 21.2% of men reported using condoms regularly, and 2.6% reported having prior chlamydial infections. Of the men that tested positive, only 13.6% reported having symptoms. Risk factors that proved useful for predicting chlamydial positivity included: African-American race, more than one sex partner, and a new sex partner. Young age was not a risk factor, as it was for women. Urine LCR is a convenient and well-accepted method for screening large numbers of men, and may be useful in developing strategies for limiting the spread of *C. trachomatis* in the population.

JANE A.
FIRST NAME

CÉCIL, M.D.
LAST NAME

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- G** To aid in the appropriate scheduling of abstracts into sessions and the compilation of the program's subject index, please list 3 key words or suggested session titles for this abstract (highest priority first). Select words in common usage, such as those used in Medline and Index Medicus.

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ABSTRACT FORM

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PREVALENCE AND RISK FACTORS OF *C. TRACHOMATIS* INFECTION IN MALE MILITARY RECRUITS: MULTIVARIATE ANALYSIS

JA Cecil, MD; MR Howell, Johns Hopkins University; JC Gaydos, MD, MPH, HM Jackson Foundation; KT McKee, MD, MPH, Ft. Bragg, NC; RM Hendrix, D.O., Ft. Jackson, SC; TC Quinn, MD, Johns Hopkins University, NIH, NIAID; CA Gaydos, DrPH, Johns Hopkins University

OBJECTIVES: To determine the prevalence and assess potential screening criteria for *Chlamydia trachomatis* infection in male military recruits, to help target future interventions.

METHODS: 1,203 male Army recruits volunteered to provide a urine sample and completed a questionnaire addressing demographic and behavioral characteristics, during inprocessing at Ft. Jackson, SC. Urine samples were tested for the presence of *C. trachomatis* using ligase chain reaction. Multivariate logistic regression was performed to access criteria for a screening program.

RESULTS: The volunteer rate was 80.9%. The prevalence of infection was 4.9%. The mean age was 19.8 years and 61.6% were Caucasian. Only 13.6% of infected men had symptoms. Independent risk factors predictive for being chlamydia positive included: African American race, having more than 1 partner in the last 90 days, and the presence of symptoms. Of 3.4% who were symptomatic, 19.5% were infected. Of asymptomatic men, 4.4% were infected. 3% of those reporting condom use were chlamydia positive.

CONCLUSION: A significant number of male Army recruits are infected with *C. trachomatis*, and the majority of these men do not have associated symptoms to suggest infection. These men may be less likely to seek appropriate medical attention, and may contribute to the transmission of chlamydial infections. Urine LCR is a convenient, noninvasive method for screening large numbers of men, and will be used to screen more recruits as part of this ongoing study. Additional data from future screening will be presented.

ABSTRACT DEADLINE:
September 21, 1998 (more on reverse)

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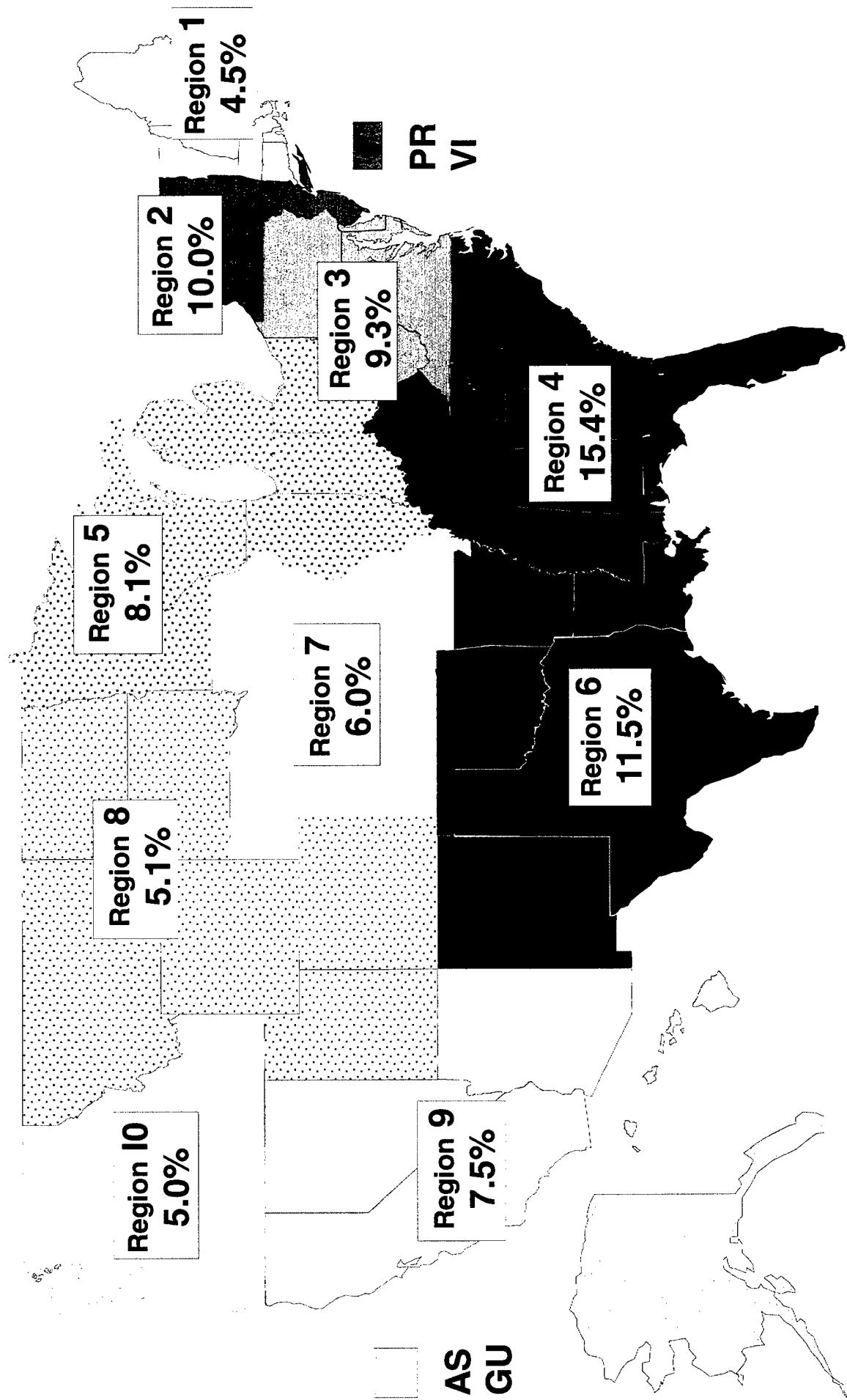
9.4 Maps

9.4.1 Percentage Chlamydia Positive Females by Geographic Origin 3rd Year

9.4.2 Percentage Chlamydia Positive Males by Geographic Origin Total

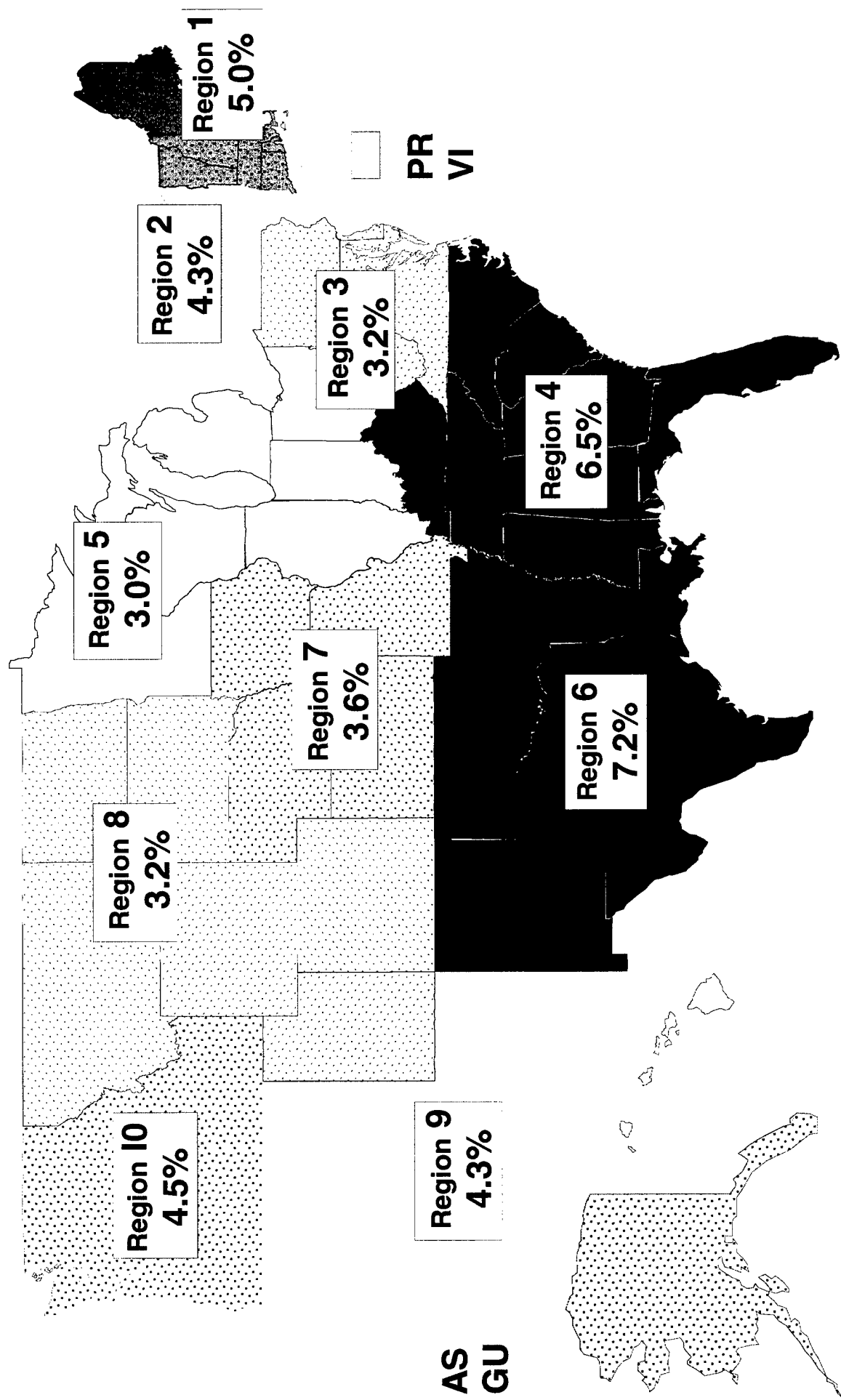
9.4.3 Percentage Chlamydia Positive Females by Geographic Origin for Project Years 1-3

Chlamydia Prevalence by Public Health Region Origin of Recruits



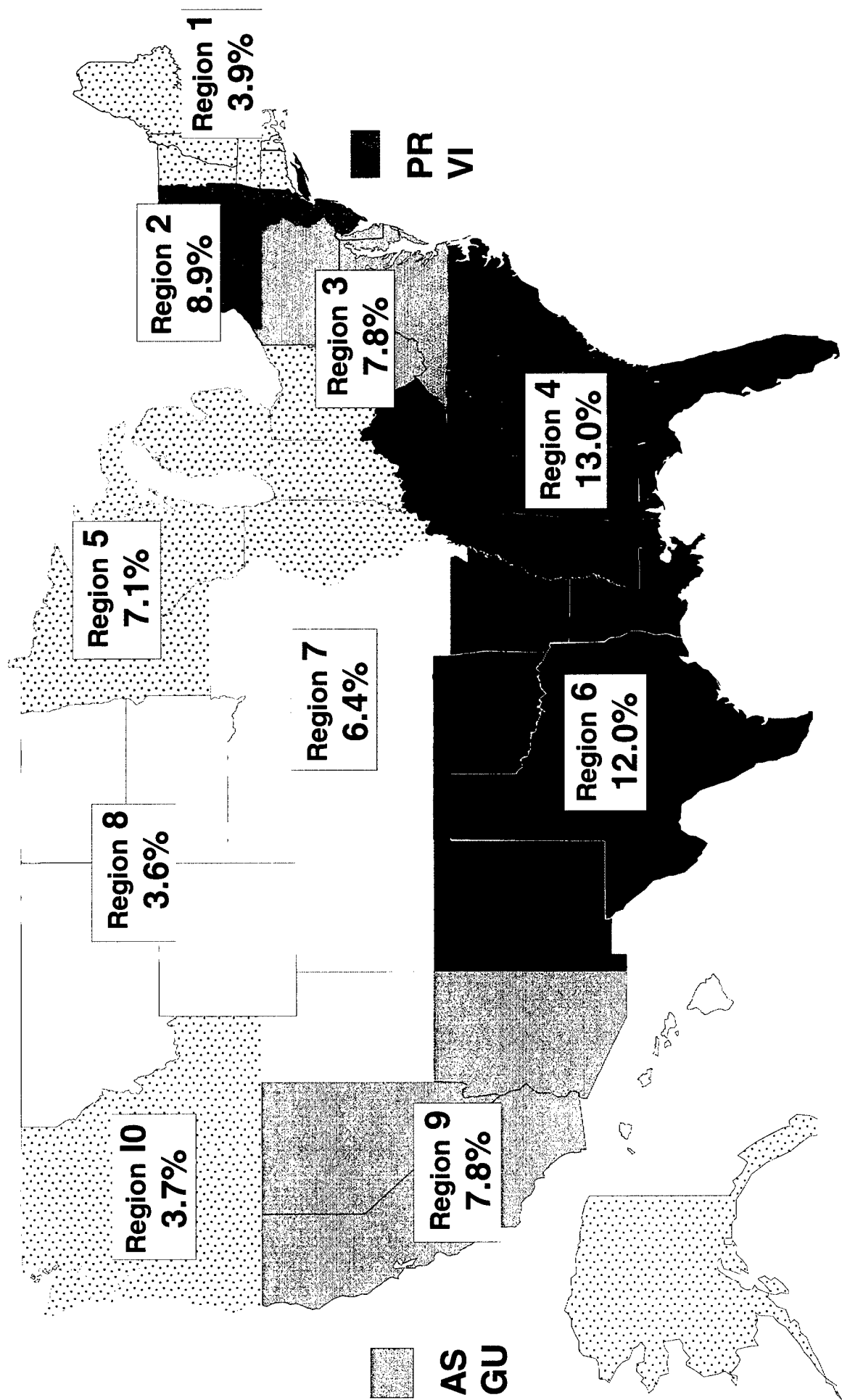
Females only (project third year)

Chlamydia Prevalence by Public Health Region Origin of Recruits



Males only (project third year)

Chlamydia Prevalence by Public Health Region Origin of Recruits



Females only (project years 1 to 3)

**9.5 Armed Forces Epidemiology Board and the Institutional Review Boards of Johns
Hopkins University and Ft. Jackson (Eisenhower, Ft. Gordon)**

MCHF-CI

9 April 1998

SUBJECT: Minutes of the Institutional Review Committee Meeting 9 April 1998

the patient was hospitalized. The patient was found to have complete heart block, systolic BP over 200mmhg, potassium level of 7.9mg/dl. She was dialyzed and a pacemaker was placed. The patient became stable and pacemaker was removed. The reporting physician felt that hyperkalemia was related to therapy with Losartan or control. The report dated 26 Jan 98 involved a 68 year old male patient who had a history of asthma and was hospitalized for asthma. The patient recovered and was discharged from the hospital. The reporter felt that the asthma was possibly related to study drug therapy. Four patients have been enrolled in this study at EAMC. There has been an adverse event at this site for ruled-out MI.

Recommendation: Full Committee Acknowledges

The Informed Consent dated 11 Sep 97 was submitted to delete the following statement, "If you are male, you should avoid fathering children by using condoms when having sexual intercourse unless you have had a vasectomy". The legal department of Merck, Inc requested the removal of this statement.

Recommendation: Approve

Human Use Committee Vote: 10 in favor, unanimous

5. Protocol Review:

Study of Chlamydia Trachomatis in Military Women: Prevalence, Risk Factors and a Cost Benefit Analysis of Early Diagnosis and Treatment
PI: LTC Rose M. Hendrix, MC
DDEAMC 95-17

With regard to Minutes of the Institutional Review Committee Meeting 12 March 1998, the investigator offered the following replies to questions raised by the committee: We propose that not only will the mass therapy option cure chlamydia, but a significant proportion of the additional cases of gonorrhea, and respiratory infections as supported above. The cost of hospitalized pelvic inflammatory disease and ectopic pregnancy are staggering for the military. The prevention of these sequelae diseases and their associated costs is the focus of this proposal. The results of a cost effective decision model we have performed has indicated that the most cost-effective strategy for the Army would be mass therapy, mostly because the diagnostic test is not perfect and the extra infections cured and sequelae prevented offset the extra cost of antibiotic. Anecdotal data from the current study has indicated that in over 1,000 women treated with single-dose azithromycin, there has only been one adverse event that caused a recruit to seek medical attention. It was not classified as serious. Further, a tabulation of 6 studies published from 1992-1998 substantiates a lack of significant differences for serious adverse episodes between individuals treated with azithromycin therapy compared to those receiving doxycycline. Reactions were considered mild to moderate and were noted primarily to be gastrointestinal in nature (diarrhea, nausea, etc). Additionally, use of mass therapy with azithromycin in a large trial (12,000 volunteers; 6,000 each of treatment arm and control arm) in Uganda has not reported any serious adverse events. The 6,000 volunteers have been treated four times. Confusion relating to misclassification of azithromycin as a macrolide (e.g., erythromycin) is frequent. The issue of pregnancy should be of no impact upon this protocol. Upon in-processing at Ft. Jackson, recruits are screened for possible pregnancy and pregnant women are not allowed to continue in basic training (EPTS). Azithromycin is a class B drug and is currently being given in clinical practice to pregnant patients. A modification of the consent form has been appended to clarify that there are no benefits to participating in this study for an individual who has never engaged in sexual activity

AY-06-98 08:20 PHYLLIS FERGUSON

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P.06

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It is well known in behavior research that there exists a high probability for a respondent to misrepresent herself. In this study we have documented 14 chlamydia infections in females who had denied sexual activity. Additionally, in analyzing demographic information on individuals choosing to not participate, it is clear the only significant difference between that group and those participating is in engagement in sexual activity. Any woman engaging in even a single episode of unprotected sexual activity in the past, can be at risk for asymptomatic disease or its sequelae. Although evidence is accumulating that there are no serious drug interactions, the investigators will ask recruits to list medications they are taking if they participate. This information will be carefully studied and tabulated. They think the consent form, which is the routine Army consent form DA Form 5303-R is clear on this point. However, they will instruct the civilian study nurse to reiterate that participation is entirely voluntary. The study nurse reports that when recruits talk to her afterwards they express concern to know whether they are infected since most chlamydial infections are asymptomatic in women. PID, ectopic pregnancy, and infertility are valid concerns of these women. They consider that the high volunteer rate is a reflection of the nurses' excellent teaching skills regarding the potential serious sequelae. (Probability of developing PID after chlamydial infection is 30% Howell et al). The recruits' desire to know about their health pales in comparisons to the ease of the collection of urine specimen.

The committee reviewed the submitted responses to its questions from the 12 March 98 meeting. Azithromycin is, in fact, an azalide antibiotic which is a subclass of macrolides structurally, but possess a very different milder side effects profile. The principal argument in favor seems to be an economic one. Based on the assumptions of the model, fewer cases of PID or asymptomatic chlamydia would result. The assumptions were challenged that a single dose could have such lasting effects. The training dollars lost could probably not be detailed that precisely. True, EPTS discharges occur but often for undiagnosed abdominal pain and not proven PID. Some are for endometriosis and other menstrually related dysmenorrhea. Silent chlamydia would show largely during an infertility workup. The need for recycling in training as a result of PID seemed high to members who have been or are currently involved in AIT and basic training. The basis for some of those lost training dollars was questioned.

The principal reason for doing the study would be for a potential policy for future basic trainees. If women (or men) were forced to take an antibiotic and developed a severe side effect, they might have a basis for a future claim against the government despite the current rule against suit by active duty members. The trend in medicine in antibiotic usage is against most prophylactic use except in very limited settings of respiratory or unavoidable threat. Otherwise disease is tested for and then treated, especially when a relatively good test is available. It is only for the false negatives that an issue exists. Repeat testing at a later date might pick up some of these false negatives, but this is not part of the study design. The issue of emerging resistance patterns was also cited. Several committee members expressed the feeling that they would be personally offended at being expected to take a pill to treat a presumed STD. The ethical issues for humans is different than that of a swine herd. The Army does not require mass prophylaxis unless the environmental threat is unavoidable. The clinical standard is still to test and then treat.

The original protocol was an all female study, however, it would be useful to know how the prevalence of chlamydia in the female recruit population compares to that of males in the same population. What they have proposed is that they test approximately 1000 male volunteers following the current protocol. These males would be counseled and tested in single gender groups by the same non-military protocol nurse we are presently using for the females. Those individuals testing positive would be referred for treatment, as are the females currently. Once male volunteers have been tested, they would go back to the original protocol and test only females for the remainder of the study.

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9 April 1998

SUBJECT: Minutes of the Institutional Review Committee Meeting 9 April 1998

Recommendation: Approve the male prevalence testing arm and correct minor errors in consent form

Clinical Investigation Committee Vote: 10 in favor, unanimous

Human Use Committee Vote: 10 in favor, unanimous

Recommendation: Disapprove the mass treatment arm.

Clinical Investigation Committee Vote: 10 in favor, unanimous

Human Use Committee Vote: 10 in favor, unanimous

6. Amendments:

A Randomized, Double-Blind, Multicenter comparison to the Efficacy and Safety of Grepafloxacin (Raxar) 400mg or 600mg Once Daily and Clarithromycin (Biaxin) 500mg Twice Daily in the Treatment of Patients with Acute Bacterial Exacerbations of Chronic Bronchitis
PI: LTC Warren L. Whitlock, MC
DDEAMC 98-17

A letter from Pharmaceutical Research Associated, Inc dated 3 Mar 98 was received regarding an amendment to this study. This amendment changes from patients with known moderate or severe hepatic or renal disease to hepatic failure or known moderate to severe renal disease. It also changes the primary efficacy population from patients who are clinically evaluable to clinically and bacteriologically evaluable. And therefore, changing the secondary efficacy population from bacteriologically evaluable to clinically evaluable. The third change in the amendment changes the package inserts for Biaxin and Raxar.

Recommendation: Approve

Human Use Committee Vote: 10 in favor, unanimous

7. Periodic Review:

A Randomized, Multicenter, Third Party Blinded Trial Comparing Trovafloxacin with Amoxicillin/Clavulanate (Augmentin) with or without Erythromycin for the Treatment of Community Acquired Pneumonia
PI: LTC Warren L. Whitlock, MC
DDEAMC 97-26

There has been good response to treatments with this drug. Trovafloxacin has now been approved by the FDA to treat Community Acquired Pneumonia and we have been part of that. A total of 16 patients have been enrolled and only one serious adverse event has been reported.

Recommendation: Approve

Human Use Committee Vote: 10 in favor, unanimous

Use of Ligase Chain Reaction with Urine versus Cervical Culture for Detection of *Chlamydia trachomatis* in an Asymptomatic Military Population of Pregnant and Nonpregnant Females Attending Papanicolaou Smear Clinics

CHARLOTTE A. GAYDOS,^{1*} M. RENE HOWELL,¹ THOMAS C. QUINN,^{1,2}
JOEL C. GAYDOS,^{3†} AND KELLY T. MCKEE, JR.⁴

Infectious Disease Division, The Johns Hopkins University, Baltimore,¹ National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda,² and U.S. Army Center for Health Promotion and Preventive Medicine, Aberdeen Proving Ground,³ Maryland, and Preventive Medicine Service, Womack Army Medical Center, Fort Bragg, North Carolina⁴

Received 13 November 1997/Returned for modification 27 January 1998/Accepted 16 February 1998

Ligase chain reaction (LCR) (Abbott Laboratories, Abbott Park, Ill.) with first-catch urine specimens was used to detect *Chlamydia trachomatis* infections in 465 asymptomatic military women attending clinics for routine Papanicolaou smear tests. Results were compared to results of cervical culture to determine the sensitivity of the urine LCR and the possible presence of inhibitors of amplification in pregnant and nonpregnant women. Discrepant results for LCR and culture were resolved by direct fluorescent antibody staining of culture sediments, two different PCR assays, and LCR for the outer membrane protein 1 gene. The prevalence of *Chlamydia* in specimens by urine LCR was 7.3% compared to 5% by culture. For 434 women with matching specimens, there were 11 more specimens positive by LCR than were positive by culture, of which all but one were determined to be true positives. There were four culture-positive, LCR-negative specimens, all from nonpregnant women. The sensitivity, specificity, and positive and negative predictive values of urine LCR after discrepant results were resolved were 88.6, 99.7, 96.9, and 99.0%, respectively. The sensitivity of culture was 71.4%. From the 148 pregnant women (prevalence by LCR, 6.8%), there were no patients who were cervical culture positive and urine LCR negative to indicate the presence in pregnant women of inhibitors of LCR. Additionally, a subset of 55 of the LCR-negative frozen urine specimens from pregnant women that had been previously processed in LCR buffer were inoculated with 5 cell culture inclusion forming units of *C. trachomatis* each and retested by LCR; all tested positive, indicating the absence of inhibitors of LCR in urine from these pregnant women. The use of LCR testing of urine specimens from asymptomatic women, whether pregnant or not, offers a sensitive and easy method to detect *C. trachomatis* infection in women.

Approximately 4 million *Chlamydia trachomatis* urogenital infections occur in the United States annually, and more than 50 million cases occur worldwide (7, 28). Unfortunately, symptoms are often mild or absent among infected men and women, leaving a large reservoir of infected persons to continue transmission to new sex partners (29). Chlamydial infections occur primarily among young sexually active persons. A high prevalence is common to all socioeconomic groups and may range from 5 to 20% in various groups of young adults (32, 33). Because of the high probability of progression of asymptomatic disease to serious sequelae, it has been recommended that individuals at risk for chlamydial infections be screened, especially women who are vulnerable to the serious consequences of genital infections, such as pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (7, 11). Urine can now be used to detect chlamydial infections in women by ligase chain reaction (LCR) (2, 8, 14, 20, 31, 34), which with its easily obtained specimen is a cost-effective method for screening programs for asymptomatic women (16). Because asymptomatic military populations have not been studied widely with

regard to chlamydial infections (4, 6, 10, 21, 26, 27) and because the sensitivity of the urine LCR assay has been reported to be low for samples from pregnant women due to the presence of inhibitors to amplification (18), we compared urine LCR to cervical culture for the detection of *C. trachomatis* in asymptomatic women attending clinics for routine Papanicolaou (PAP) smear tests.

MATERIALS AND METHODS

Populations and specimens. Military women ($n = 480$) attending clinics for a routine PAP smear test volunteered for a study to compare urine LCR tests to cervical cultures for the detection of *C. trachomatis* infections. The volunteer rate of the women approached by the civilian research nurse was 71%. The study was approved by the Institutional Review Boards of The Johns Hopkins University, the U.S. Army Medical Research Materiel Command, Fort Detrick, Frederick, Md., and Womack Army Medical Center, Fort Bragg, N.C. Of 480 women enrolled, 465 provided a urine specimen. All subjects completed a questionnaire for demographic information and behavioral risk factors for sexually transmitted diseases. The data instrument was a one-page, two-sided scanable bubble form (Scantron Corporation, Tustin, Calif.). During the pelvic examination, an endocervical swab was obtained by the attending clinician at the PAP smear clinic, who recorded clinical signs and symptoms on the data form. Culture swabs were placed into 2-sucrose-phosphate chlamydia transport medium. Commercial transport medium was replaced with in-house transport medium after 1 month of the study due to some toxicity of the former to tissue culture cells. Specimens were stored appropriately (4°C for urine specimens and -70°C for cultures) until shipping of the urine specimens at 4°C and cultures at -70°C. Shipments were made to ensure arrival at the laboratory within 4 days of collection. All specimens, consent forms, and data forms were shipped to Johns Hopkins Chlamydia Research Laboratory.

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Laboratory procedures. Urine specimens were processed and tested by LCR (Abbott Laboratories, Abbott Park, Ill.) according to the manufacturer's instructions. Briefly, 1 ml of urine was centrifuged at $15,000 \times g$ for 15 min. After the supernatant was removed, 1 ml of urine buffer was added to the pellet and the mixture was vortexed. After being heated at 97°C for 15 min, specimens were cooled and 100 μl of each specimen was added to an LCR unit dose tube. An appropriate chlamydia-positive control was included for the processing steps for each group of specimens. Additionally, two negative controls and two positive calibrator controls supplied by the manufacturer were used for each LCR assay run. After the amplification step in the automated thermocycler, unit dose tubes containing the specimens and controls were transferred to the automated enzyme immunoassay machine for the detection of amplified products. Tubes containing the amplified products were never opened; the automated enzyme immunoassay process sampled tubes by piercing the tops of the unit dose tubes, which prevented amplicon contamination. In order to prevent other sources of contamination, specimens were processed in a designated room separate from the room used to amplify and detect specimens. Gloves were frequently changed and aerosol-barrier pipette tips and dedicated pipettors were used. Strict quality-control measures such as machine maintenance checks, daily cleaning of laboratory areas and equipment with bleach, and area swipe tests to monitor amplicon contamination were employed.

Culture specimens were stored frozen at -70°C for up to 3 days. Cultures were done in 96-well microwell plates in McCoy cells by standard methods (12). Tissue cultures were stained with genus-specific fluorescein-conjugated antibody (Kallested, Chaska, Minn.) and species-specific antibody (Boehringer Mannheim/Syva, San Jose, Calif.). Stained cultures were read for the presence of chlamydial inclusion bodies with an epifluorescence microscope.

Discrepancy analysis was done for any sample with discordant results between culture and LCR. A sample that was positive by culture and negative by LCR was considered to be a true positive, but the discrepancy was investigated for the presence of inhibitors to amplification by LCR. The urine LCR was repeated from the originally processed specimen and repeated again after diluting the processed specimen 1:10 in urine LCR buffer to check for the presence of inhibitors in the specimen. (Dilution has been shown to sometimes decrease the concentration of the inhibitor enough to allow a true-positive specimen to be amplified.) Additionally, PCR (Roche Diagnostic Systems, Branchburg, N.J.) was done on an archived aliquot of frozen urine and another LCR was done for a different DNA target, the outer membrane protein 1 (OMP-1) gene. For specimens that were positive by LCR and negative by culture, the culture specimen transport sediment was stained by direct fluorescent antibody (DFA) (Boehringer Mannheim/Syva) for chlamydial elementary bodies. PCR also was done on the specimens from the culture transport vials. In addition, PCR was done on the archived urine and an LCR for the OMP-1 gene was done on the previously processed (buffered) urine specimen. Specimens that were positive by one or more of the ancillary tests were considered true positives. An LCR-positive urine specimen which could not be confirmed by another test was considered to be a false positive.

Testing of urine specimens from pregnant women. A subset of all available ($n = 55$) previously processed (buffered) LCR-negative urine specimens that were from pregnant women were inoculated with 5 inclusion forming units of *C. trachomatis* and retested by LCR to check for the presence of inhibitors. Additionally, 65 archived LCR-negative unprocessed urine specimens that were available from pregnant women were tested by a research internal control assay to evaluate the presence of inhibitors (9). This assay tested for the ability to amplify an extraneous sequence of DNA which was added as an internal control to the specimen. The assay contained primers for the extraneous DNA internal control as well as the primers for the organism of interest. A positive amplification of the internal control indicated that the specimen contained no inhibitors to the amplification process, while a negative result indicated that the specimen contained something which inhibited the amplification process.

Data analysis. The data from the questionnaire forms were scanned into a data set (D-base III Plus; Ashton Tate, Borland International, Spring Valley, Calif.), and LCR results, demographics, and risk factor information were analyzed by the chi-square test, Fisher's tests of exactness, and univariate analysis (Intercooled Stata, version 4.0; Stata Corporation, College Station, Tex.).

RESULTS

Patient characteristics. Among the 480 women enrolled, only 1 woman had reported mild symptoms and the remainder were asymptomatic. Approximately half (55.2%) were 25 years or younger, and 50.8% were African-American. Over 90% were enlisted personnel, 98.3% reported vaginal sex, 11.3% had a new sex partner in the previous 90 days, 15.2% had more than one sex partner in the previous 90 days, 88.5% reported inconsistent condom use, and 30.8% were pregnant (Table 1). Reasons for clinic visit, clinical presentation, and sexual risk history are presented in Table 1. Of the 465 women who provided a urine specimen, the overall prevalence for chla-

TABLE 1. Characteristics of military women screened for *C. trachomatis* at Fort Bragg, N.C.

Variable	No. of women ($n = 480$)	%
Median age, yr (range) ^a	25 (19-47)	
Ethnicity ^b		
White	181	37.7
African-American	244	50.8
Other (American Indian, Alaskan, or Asian Pacific)	52	10.8
Military category ^c		
Enlisted personnel	439	91.5
Officer	36	7.5
Reason for clinic visit ^d		
Sex partner of infected individual	1	0.2
Complaint of symptoms	1	0.2
Screening	470	97.8
Other	4	0.8
Clinical presentation ^d		
Mucopus	1	0.2
Cervicitis	5	1.0
Ectopy	5	1.0
Cervical motion tenderness	3	0.6
Friability	39	8.1
Pregnant	148	30.8
Normal exam	348	72.5
Sexual history (past 90 days)		
More than one sex partner	73	15.2
New sex partner	54	11.3
Consistent condom use	51	10.6
Inconsistent condom use	425	88.5
Previous diagnosis ^e		
<i>N. gonorrhoeae</i>	20	4.2
<i>C. trachomatis</i>	86	17.9
Syphilis	5	1.0
Trichomonas	41	8.5
None	348	72.5
Chlamydia-positive urine LCR ^f	34	7.3
Chlamydia-positive culture ^g	24	5.3

^a Data missing for eight women (1.7%).

^b Data missing for three women (0.6%).

^c Data missing for five women (1.0%).

^d Data missing for 27 women (5.6%).

^e Data missing for two women (0.4%).

^f Urine specimen for LCR missing for 15 women.

^g Cervical specimens for culture missing for 21 women and 10 toxic specimens collected.

mydia infection by LCR was 7.3%. The prevalences of infection for other categories based on LCR included 11.0% for women ≤ 25 years of age, 8.9% for African-American women, and 6.8% for pregnant women. By risk category the prevalences were 15.1% for those with a new sex partner in the previous 90 days, 10.3% for those with more than one sex partner in the previous 90 days, 7.5% for those with inconsistent condom use, 7.4% for those reporting vaginal sex, and 3.6% for those with a prior chlamydial infection.

In univariate analysis only young age (≤ 25 years) (odds ratio [OR], 4.23; 95% confidence interval [CI], 1.72 to 10.43) and a new sex partner (OR, 2.61; 95% CI, 1.11 to 6.1) were predictors of chlamydial infection (Table 2). However, when we controlled for age, a new sex partner was no longer significant.

Comparison of urine LCR to cervical culture. Of the 465 women, 31 women did not have matched culture specimen results. Ten specimens were toxic for tissue culture and no cervical cultures were collected from 21 women, leaving 434 matched specimens for comparison. After the use of the commercial chlamydia transport buffer was stopped and the in-

TABLE 2. Univariate analysis of results relative to factors associated with positive urine LCRs for military women attending PAP smear clinics

Factor ^a	% with a positive LCR		OR (95% CI)	P
	Factor absent	Factor present		
Age ≤25 yr (254)	2.8	11.0	4.2 (1.72, 10.43)	0.002
African-American (233)	6.8	8.6	1.3 (0.61, 2.7)	0.501
Pregnant (142)	7.4	7.0	0.94 (0.44, 2.03)	0.882
Normal pelvic exam (275)	12.5	6.6	0.49 (0.18, 1.31)	0.154
Prior diagnosis of STD ^b (127)	8.3	3.9	0.45 (0.17, 1.2)	0.110
Having had more than one sex partner in last 90 days (68)	6.8	10.3	1.6 (1.53, 3.73)	0.316
Having had a new sex partner in last 90 days (53)	6.4	15.1	2.6 (1.11, 6.10)	0.027 ^c

^a Numbers in parentheses represent numbers of individuals with the factor present ($n = 465$).

^b STD, sexually transmitted disease (chlamydia, gonorrhea, syphilis, or trichomonas).

^c A new sex partner was not significant when we controlled for age.

house 2-sucrose-phosphate medium was used, no further specimens toxic to tissue culture were observed. Among the 31 specimens without matched results, there were two LCR-positive urine specimens for which a matching cervical culture was not collected.

From the 434 matched specimens, 32 (7.4%) were LCR positive, of which 31 (7.3%) were confirmed as true positives (Table 3). There were 21 LCR-positive, culture-positive specimens. Four patients had urine-LCR-negative, cervical-culture-positive specimens. Discrepancy analysis of these LCR-negative, culture-positive specimens demonstrated that one was positive in the repeat LCR assay and was OMP-1 LCR positive, one had a negative value which was close to the cutoff value for a positive result and was PCR positive when the archived frozen urine was tested, one had a culture transport specimen that was PCR positive, and the results of one could not be confirmed by any of the ancillary tests, including repeat culture. The initial LCR-negative results from these four urine specimens were all considered to be false negatives.

There were 11 specimens that were LCR positive and culture negative, 10 of which could be confirmed as true-positive specimens (Table 4). Five were DFA positive, six were urine PCR positive, seven were culture PCR positive, and eight were OMP-1 LCR positive. Thus, all but one of these LCR-positive specimens were confirmed as true positives by at least one or more additional assays. After resolution of the discrepant results, the sensitivity, specificity, and positive and negative predictive values of urine LCR were 88.6, 99.7, 96.9, and 99.0%, respectively (Table 3), and the sensitivity of culture was 71.4%.

TABLE 3. Comparison of urine LCR to cervical culture for *C. trachomatis* in military women attending PAP smear clinics

Test	Result	No. of women ^a with test result (%)	Resolved patient infection status ^b	
			Positive	Negative
Cervical culture	Positive	25 (5.8)	25	0
	Negative	409 (94.2)	10	399
Urine LCR ^c	Positive	32 (7.4)	31	1
	Negative	402 (92.6)	4	398

^a Four hundred eighty women enrolled; 434 had matching specimens.

^b Resolved patient infection status was defined as the resolved status of a patient with a positive culture or with a urine specimen positive by two tests (LCR, DFA staining, PCR OMP-1 gene, PCR plasmid gene, and LCR OMP-1).

^c After discrepant results were resolved, sensitivity was 88.6%, specificity was 99.7%, and positive and negative predictive values were 96.9% and 99.0%, respectively (sensitivity of culture, 71.4%).

LCR of urine of pregnant women. There were 148 urine specimens from pregnant women. The prevalence of chlamydia infection by LCR for the pregnant women was 6.8%, and that for the nonpregnant women was 7.8%. There were no culture-positive, LCR-negative results from pregnant women which could have indicated the presence of LCR inhibitors. All four of the culture-positive, LCR-negative specimens were from women who were not pregnant. In addition, a subset of 55 LCR-negative urine specimens, previously processed in LCR buffer and frozen, which were from pregnant women and were inoculated with chlamydia and retested by LCR were all LCR positive, indicating the lack of inhibitors. Of the 65 available archived urine specimens from pregnant women which were LCR negative and tested in the internal control assay, there were 3 (4.6%) that exhibited inhibition based on a negative value for amplification of the internal control.

DISCUSSION

Chlamydia infections were of a higher prevalence than expected from these asymptomatic military women attending a clinic for a routine PAP smear test. An LCR prevalence of 7.3% underscores the necessity for the recommendation to screen all sexually active young women when they are attending a routine health care clinic (7). The high prevalence of 11.0% for those ≤25 years of age confirm the result of studies of others that young age is a significant risk factor for chlamydial infections (13, 17, 22). These results indicate the need

TABLE 4. Resolution of urine-LCR-positive and cervical-culture-negative discrepant results for *C. trachomatis* in military women attending PAP smear clinics ($n = 11$)

Laboratory no.	Test result					Status
	LCR (urine)	DFA ^a	PCR (urine)	PCR (cervix)	LCR for OMP-1 (urine)	
1264	+	—	—	—	+	Confirmed
2407	+	+	+	+	+	Confirmed
3197	+	+	+	+	+	Confirmed
3659	+	—	—	+	—	Confirmed
3891	+	—	—	—	+	Confirmed
5560	+	+	—	+	+	Confirmed
5570	+	—	—	—	—	Unconfirmed
6082	+	—	+	+	+	Confirmed
6280	+	+	+	+	—	Confirmed
6966	+	+	+	+	+	Confirmed
8016	+	—	+	—	+	Confirmed

^a DFA staining of culture transport vial specimen.

for an ongoing chlamydial control program for such female military personnel as those enrolled in this study. This population demonstrated a high degree of sexual behaviors placing them at risk for sexually transmitted diseases, with 98% being sexually active, 15% having more than one partner, 11% having a new partner in the last 90 days, and 88% using condoms inconsistently. All of these behaviors have been shown by others to be predictive of chlamydial infection (1, 22–24, 36). In the univariate analysis for this study, both young age (prevalence, 11.0%) and having had a new partner (prevalence, 15.1%) reached statistical significance. However, when we controlled for age, a new sex partner was not significant. Young age (≥ 25 years), which is an easily determined risk factor and which is a nonthreatening question for those women who may be reticent to answer questions about their sexual behavior, appears to be an excellent predictor of chlamydia infection and can be recommended for deciding who should be screened in clinical or outreach situations (13, 17).

Urine LCR performed well in this study of asymptomatic women, with a sensitivity of 88.6%, which is similar to that demonstrated by others for asymptomatic women (87.5%) (2). Compared to cervical culture, which had a sensitivity of 71.4%, LCR detected more infected women. Many reasons can account for the lower culture sensitivity. Not only can the cold chain of transport be interrupted, but the quality of the transport medium is important as well. Initially, a commercially available transport medium was used in this study, which resulted in many (10) toxic tissue culture results. Quality-control assays of the remaining lot of uninoculated transport medium demonstrated that it was toxic to cells in tissue culture. After switching to the use of our own transport medium, which is quality controlled in tissue culture, we observed no further toxicity.

Additionally, the quality of the endocervical specimen, as measured by the presence of columnar epithelial cells, has been shown to play a significant role in the numbers of positive specimens (19, 37). In another study of family-planning clinics in Baltimore, Md., clinicians obtained adequate specimens only 72.3% of the time (37). Thus, inadequate cervical swab specimens could have contributed to the lower sensitivity of culture in our study. Other studies have demonstrated higher sensitivities for urine LCR than cervical culture (2, 5, 8, 20, 31, 34). Sensitivities for cervical culture in these studies has ranged from 45.5 to 46.9% to 55.6 to 65.0% (2, 5, 8, 34). Schachter et al. have demonstrated that the sensitivity of culture for *C. trachomatis* may be increased from 67.1% to 74% by adding a urethral swab culture, which could be indicative that some women may be infected only in the urethra and not the cervix (31). This could help explain the higher number of positives found by urine LCR, presumably reflecting infections from both the cervix and the urethra. Because urine is an easy-to-obtain, noninvasive specimen giving accurate results with LCR, it is ideal for screening asymptomatic individuals who may not be presenting for a pelvic exam or for outreach screening programs.

Although our study enrolled only 148 women who were pregnant, we did not observe any indication of inhibitors in urine specimens, as evidenced by the lack of urine-LCR-negative results when the cervical culture was positive. Although there were four such specimens in this study, they were all from nonpregnant women. Another study has reported a significant problem with inhibitors in urine with use of the LCR test; however, the urine specimens were transported at ambient temperatures, which may have influenced the LCR results (18, 25, 30). The spiking experiment in our study did not demonstrate any inhibitors in the 55 LCR-negative, previously frozen

urine specimens from pregnant women. It is possible that freezing and thawing of these processed urine specimens reduced or destroyed some LCR inhibitors. Freezing and thawing reduced the inhibition from 19 to 16% in one study (35). Additionally, the experiment which tested the archived urine of 65 pregnant women demonstrated only three (4.6%) inhibited specimens. This value is of the same order of magnitude as that reported by others for inhibition in urine specimens (2.6 and 1.8%) for amplified testing (3, 15). Most investigators now believe that inhibitors to amplification exist for both urine and cervical specimens (3, 15, 35). A combination of heat treatment (95°C for 10 min) and 10-fold dilution of the processed specimen reduced inhibition of PCR from 19 to 4% in one study (35). The pH of the cervical mucosa was partly correlated with inhibitors (35). Decreased inhibition was found at pH values of ≥ 7.5 . The degree to which inhibitors to amplification influence the prevalence detected by LCR and PCR needs to be further studied. Roche Molecular Systems has addressed this problem by incorporating an internal DNA control amplification and detection assay into their new combination PCR assay for *C. trachomatis* and *Neisseria gonorrhoeae*, which will prove to be a great advance in the diagnostic capability of amplification assays. Specimens exhibiting inhibitors can be diluted or heated and their DNA can be extracted, and tests can be repeated. The use of the internal control will give a greater degree of confidence to the validity of a negative amplification result. Consideration of the use of an internal control should be given for amplification tests in the future. The College of American Pathologists now requires examination of a control to assess the presence of inhibitors in all amplification procedures.

In summary, young sexually active women, including those in the military, should be frequently screened for chlamydia infections. Urine LCR offers an easy and sensitive method to accomplish this, especially for women not presenting for a pelvic examination. It is cost-effective in preventing the expensive sequelae of pelvic inflammatory disease, ectopic pregnancy, and tubal infertility (16).

ACKNOWLEDGMENTS

We thank the study coordinator, Barbara Pare; the research nurses Eleanor Howard, Katy Cline, and Bobbi Jones and the staffs of the Fort Bragg clinical sites for obtaining specimens; the laboratory technicians Graciela Jaschek, Laura Welsh, Dien Pham, Diana Perkins, Sandy Leister, and Kimberly Crotchfeld for performance of laboratory tests and data entry; Kathryn Clark for statistical assistance; and Pat Buist for assistance in manuscript preparation.

Funding for this study was from Department of the Army grant DAMD 17-95-1-5064.

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The New England Journal of Medicine

Established in 1812 as THE NEW ENGLAND JOURNAL OF MEDICINE AND SURGERY

VOLUME 339

SEPTEMBER 10, 1998

NUMBER 11

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THE NEW ENGLAND JOURNAL OF MEDICINE (ISSN 0028-4793) is published weekly from editorial offices at 10 Shattuck Street, Boston, MA 02115-6094. Subscription price: \$122.00 per year. Periodicals postage paid at Boston and at additional mailing offices. POSTMASTER: Send address changes to P.O. Box 803, Waltham, MA 02454-0803.

CHLAMYDIA TRACHOMATIS INFECTIONS IN FEMALE MILITARY RECRUITS

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ABSTRACT

Background Asymptomatic genital *Chlamydia trachomatis* infections in women can lead to pelvic inflammatory disease, infertility, and ectopic pregnancy. To design a chlamydia-control program, we conducted a large survey of women in the U.S. military.

Methods From January 1996 through December 1997, urine samples from 13,204 new female U.S. Army recruits from 50 states were screened by ligase chain reaction for *C. trachomatis* infection. Information on potential risk factors was obtained by questionnaire. With multivariate analysis, we identified criteria for a screening program.

Results The overall prevalence of chlamydial infection was 9.2 percent, with a peak of 12.2 percent among the 17-year-old recruits. The prevalence was 15 percent or more among the recruits from five southern states. The following risk factors were independently associated with chlamydial infection: having ever had vaginal sex (odds ratio for infection, 5.9), being 25 years of age or less (odds ratio, 3.0), being black (odds ratio, 3.4), having had more than one sex partner in the previous 90 days (odds ratio, 1.4), having had a new partner in the previous 90 days (odds ratio, 1.3), having had a partner in the previous 90 days who did not always use condoms (odds ratio, 1.4), and having ever had a sexually transmitted disease (odds ratio, 1.2). A screening program for subjects 25 years of age or less (87.9 percent of our sample) would have identified 95.3 percent of the infected women.

Conclusions Among female military recruits, the prevalence of chlamydial infection is high. A control program that screens female recruits who are 25 years old or younger with urine DNA-amplification assays has the potential to reduce infection, transmission, and the sequelae of chlamydial infection. (N Engl J Med 1998;339:739-44.)

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MORE than 4 million urogenital *Chlamydia trachomatis* infections occur in the United States annually.^{1,2} They occur in young, sexually active persons from all socioeconomic groups, with prevalence ranging from 5 percent to 20 percent.^{3,4} Women, especially, bear the burden of disease, with consequences of genital infections ranging from pelvic inflammatory disease to ectopic pregnancy and infertility.^{1,5} These sequelae are associated with a large economic burden.^{6,7} Because up to 80 percent of infected women are asymptomatic and therefore do not seek

medical care, screening of young, sexually active women has been recommended.^{1,8} In the past, screening for *C. trachomatis* infections in women has been limited by the need for access to a medical clinic and a pelvic examination. However, *C. trachomatis* infections can now be detected with high sensitivity (85 to 95 percent) and specificity with DNA-amplification assays performed on urine specimens,⁹⁻¹⁴ allowing cost-effective screening of large numbers of women in nonclinic settings.¹⁵

Few studies of the prevalence of chlamydial infection in U.S. military populations have been published, and there have been no studies using DNA-amplification techniques among women not seeking health care.¹⁶⁻²⁰ Because adolescents have the highest prevalence of disease and most military recruits are young, we conducted a large prevalence study and risk-factor analysis of female recruits from throughout the United States who began basic training at Fort Jackson, South Carolina. We performed this study to determine the extent of infection, assess the feasibility of screening urine specimens for *C. trachomatis* by the ligase chain reaction, and assess which epidemiologic correlates would be useful for implementing an effective chlamydia-control program for female recruits.

METHODS

Population and Specimens

All female Army recruits who were present on Sundays between January 1996 and December 1997 at the Physical Examination Section, Reception Battalion, Fort Jackson, South Carolina, were invited to participate in this study. The study was approved by the institutional review boards of Johns Hopkins University and Fort Jackson (Eisenhower Army Medical Center, Fort Gordon, Ga.), as well as the Human Subjects Research Review Board of the U.S. Army Surgeon General. Of the 16,593 recruits approached, 13,223 (79.7 percent) volunteered to participate in the study and were given a briefing about the study as well as an educational briefing about chlamydial infections by the civilian research nurse.

All subjects signed an informed-consent form and completed a questionnaire regarding demographic information, home state,

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and sexual history. The data instrument was a two-sided scannable form (Scantron, Tustin, Calif.). To determine the similarity of the study subjects and those who chose not to participate in the study with regard to demographic characteristics and sexual history, 823 of the 3370 women who did not volunteer were invited to fill out an anonymous questionnaire. Nonvolunteers were asked to fill out a questionnaire only during the first week of each month.

Each volunteer was instructed to collect 20 to 30 ml of first-catch urine (the first part of the urine stream). A unique study number was assigned to each volunteer. All urine specimens, consent forms, and questionnaires were shipped to the Johns Hopkins University chlamydia laboratory. Urine specimens were kept at 4°C until processed, within 48 hours.

Laboratory Procedures and Treatment

Urine specimens were processed and tested by the ligase chain reaction (Abbott Laboratories, Abbott Park, Ill.) for chlamydial DNA according to the manufacturer's directions. Each week a list of infected subjects was sent to the research nurse. The infected subjects were contacted and treated at the Troop Medical Clinic at Fort Jackson by directly observed therapy with a single 1-g dose of azithromycin. The subjects were also tested for coexisting sexually transmitted diseases. The sensitivity and specificity of the ligase chain reaction in urine specimens as compared with cervical culture for chlamydia had been previously determined to be 88.6 percent and 99.7 percent, respectively, in another military population.¹⁴

Statistical Analysis

Questionnaire forms were scanned into a data base (dBASE III Plus, Borland International, Spring Valley, Calif.). The results of the ligase chain reaction, demographic information, and risk-factor information were analyzed as dichotomous variables with the chi-square test. Univariate and multivariate logistic-regression analysis for factors associated with chlamydial infection was performed with Intercooled Stata software (version 4.0, Stata, College Station, Tex.). All independent variables were entered into the model, and a two-sided P value of less than 0.05 was considered to indicate statistical significance. The 95 percent confidence interval for the prevalence value for recruits from each state was calculated with Stata software. A one-way analysis of variance was performed to assess the degree of significance of differences in prevalence between states.

RESULTS

Characteristics of the Subjects

Of 13,223 subjects presenting at the Physical Examination Section on Sundays from January 1996 through December 1997, 19 could not be evaluated because of missing data or insufficient urine. The median age of the 13,204 who could be evaluated was 21 years (range, 17 to 39); 87.9 percent (11,603) were 25 years old or younger (Table 1). Fifty-one percent of the women were white, 35.9 percent were black, and 13.1 percent were of other races. For the entire population, the prevalence of *C. trachomatis* infection according to the urine ligase chain reaction was 9.2 percent.

On the questionnaire, 93.1 percent of the subjects reported having ever had vaginal sex, 26.7 percent having had more than one sex partner in the previous 90 days, and 31.4 percent having had a new sex partner in the previous 90 days. Only 16.9 percent reported that their partners always used condoms. A history of chlamydial infection was reported by 9.1

TABLE 1. CHARACTERISTICS OF 13,204 FEMALE ARMY RECRUITS SCREENED FOR *CHLAMYDIA TRACHOMATIS*.

CHARACTERISTIC	VALUE
Age — yr	
Median	21
Range	17–39
Race — no. (%)*	
White	6,715 (51.0)
Black	4,733 (35.9)
Other	1,726 (13.1)
Ever had vaginal sex — no. (%)†	12,281 (93.1)
Sexual history in previous 90 days — no. (%)	
More than one partner‡	3,478 (26.7)
New partner§	4,076 (31.4)
Partner always used condoms¶	2,115 (16.9)
Previous diagnosis of sexually transmitted disease — no. (%)	
<i>Chlamydia trachomatis</i>	1,206 (9.1)
<i>Neisseria gonorrhoeae</i>	430 (3.3)
Syphilis	74 (0.6)
Trichomonas	611 (4.6)
None	11,372 (86.1)
Chlamydia-positive — no. (%)	1,219 (9.2)

*Data were missing for 30 subjects.

†Data were missing for 9 subjects.

‡For 168 subjects, data were missing or subject did not know answer.

§For 225 subjects, data were missing or subject did not know answer.

¶For 684 subjects, data were missing or subject did not know answer.

percent of the subjects, gonorrhea by 3.3 percent, syphilis by 0.6 percent, and trichomonas infection by 4.6 percent. Of the volunteers who reported having had no vaginal sex, 1.4 percent (13 of 914) were chlamydia-positive, and of those who reported that their partners always used condoms, 8.4 percent (177 of 2115) were chlamydia-positive.

Of the 823 nonvolunteer recruits who filled out a questionnaire anonymously, 203 (24.7 percent) did not provide their ages and were dropped from the analysis. The mean age of the remaining nonvolunteer recruits was 21 years (range, 17 to 36); 51.3 percent were white, and 31.9 percent were black. The mean age and the racial distribution of these recruits were not significantly different from those of the volunteers. Only 66.9 percent reported having had vaginal sex, as compared with 93.1 percent of the volunteers ($P<0.001$). This group differed significantly from the volunteers in four variables, even after adjustment for whether the women reported having had vaginal sex: only 4.0 percent reported prior chlamydial infections ($P=0.013$), 18.2 percent had had a new sex partner in the previous 90 days ($P=0.002$), 20.1 percent had partners who consistently used condoms ($P<0.001$), and 90.7 percent reported no previous diagnosis of a sexually transmitted disease ($P=0.001$). Of the nonvolunteers, 17.7 percent had had more than one sex partner in the previous 90 days; the proportion of the volunteers who had had more than

one sex partner in the previous 90 days was similar after adjustment for vaginal sex ($P=0.189$).

Prevalence of Infection

The age-specific prevalence of *C. trachomatis* infection among the 13,204 volunteers is shown in Figure 1. The highest prevalence of chlamydial infection (12.2 percent) was among 17-year-olds. The prevalence declined sharply with increasing age, to below 5 percent for women over 25 years of age. For further analysis, the youngest age groups (17 to 25 years) were combined into a category called "young." The prevalence in this group was 10.0 percent (1162 of 11,603). In the older-age category (26 to 39 years), the prevalence was 3.6 percent (57 of 1601). The prevalence was 5.5 percent (369 of 6715) for whites, 14.9 percent (707 of 4733) for blacks, and 8.1 percent (143 of 1756) for other races.

Univariate Analysis

Univariate analysis identified 10 variables significantly associated with chlamydial infection: young age (17 to 25 years), black race, race other than white or black, ever having had vaginal sex, having had more than one sex partner in the previous 90 days, having had a new sex partner in the previous 90 days, having had a partner who did not always use condoms in the previous 90 days, a prior diagnosis of gonorrhea, a prior diagnosis of trichomonas, and a history of any sexually transmitted disease (Table 2). A prior diagnosis of chlamydia or syphilis was not significantly associated with being positive for chlamydial infection.

Multivariate Analysis

In the complete multivariate model, having had vaginal sex, an age of 25 years or less, black race, hav-

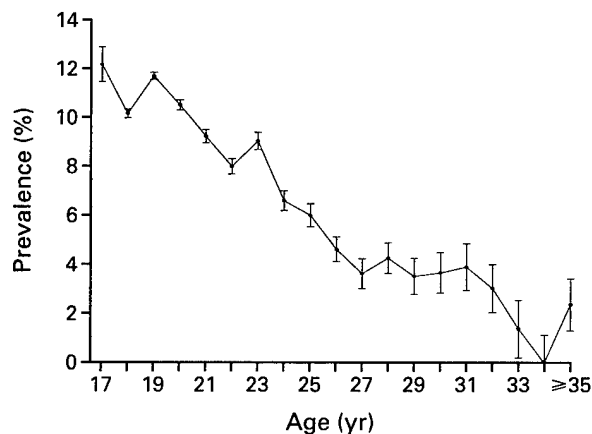


Figure 1. Mean (\pm SE) Age-Specific Prevalence of Chlamydial Infection among 13,204 Female Army Recruits, According to Ligase-Chain-Reaction Assays of Urine Specimens.

ing had more than one sex partner in the previous 90 days, having had a new sex partner in the previous 90 days, having had a partner who did not always use condoms in the previous 90 days, and a history of any sexually transmitted disease were independent predictors of chlamydial infection (Table 3).

Strategies for Selective Screening

A screening strategy involving all variables identified as independent predictors would require that 100 percent of the population be tested and would detect 100 percent of the positive subjects. In this model, the magnitude of risk associated with having had a new sex partner might vary according to race. For the purpose of a screening program, this would not alter the proportion of the population tested or the percentage of positive subjects detected with this model. Because screening on the basis of race would probably be viewed as inequitable, a strategy excluding race was examined. According to this strategy,

TABLE 2. UNIVARIATE ANALYSIS OF FACTORS ASSOCIATED WITH CHLAMYDIAL INFECTION IN FEMALE ARMY RECRUITS.*

RISK FACTOR	NO. OF RECRUITS	PREVALENCE OF INFECTION		
		RISK FACTOR PRESENT	RISK FACTOR ABSENT	ODDS RATIO (95% CI)
Age ≤ 25 yr	11,603	10.0	3.6	3.0 (2.3–4.0)
Black race†	4,733	14.9	5.5	3.0 (2.7–3.5)
Other (nonwhite, nonblack) race†	1,726	8.1	5.5	1.5 (1.2–1.9)
Having ever had vaginal sex	12,281	9.8	1.4	7.5 (4.4–13.1)
Having had >1 sex partner in previous 90 days‡	3,478	13.5	7.7	1.9 (1.7–2.1)
Having had a new sex partner in previous 90 days§	4,076	12.4	7.8	1.7 (1.5–1.9)
Having had a partner who did not always use condoms in previous 90 days¶	10,405	9.8	NA	1.2 (1.0–1.4)
Condom use unknown¶	418	5.0	NA	0.6 (0.4–0.9)
Data on condom use missing¶	266	1.5	NA	0.2 (0.1–0.5)
Previous diagnosis of <i>Neisseria gonorrhoeae</i>	430	12.3	9.1	1.4 (1.0–1.9)
Previous diagnosis of trichomonas	611	11.6	9.1	1.3 (1.0–1.7)
History of any sexually transmitted disease	1,828	10.5	9.0	1.2 (1.0–1.4)

*CI denotes confidence interval, and NA not available.

†The reference group consisted of the white subjects.

‡The variable is dichotomized: the reference group consisted of the subjects who did not have >1 sex partner, who answered that they did not know, or for whom data were missing.

§The variable is dichotomized: the reference group consisted of the subjects who did not have a new sex partner, who answered that they did not know, or for whom data were missing.

¶The variable is dichotomized: the reference group consisted of the subjects who had a partner who always used condoms.

TABLE 3. MULTIVARIATE ANALYSIS OF FACTORS INDEPENDENTLY ASSOCIATED WITH CHLAMYDIAL INFECTION IN FEMALE ARMY RECRUITS.

RISK FACTOR	ODDS RATIO (95% CI)*
Age ≤ 25 yr	3.0 (2.3–4.0)
Black race†	3.4 (2.9–3.8)
Other (nonwhite, nonblack) race†	1.7 (1.4–2.1)
Having ever had vaginal sex	5.9 (3.2–10.6)
Having had >1 sex partner in previous 90 days‡	1.4 (1.2–1.7)
Having had a new sex partner in previous 90 days§	1.3 (1.1–1.6)
Having had a partner who did not always use condoms in previous 90 days¶	1.4 (1.1–1.6)
Having ever had a sexually transmitted disease	1.2 (1.0–1.4)

*CI denotes confidence interval.

†The reference group consisted of the white subjects.

‡The reference group consisted of the subjects who did not have >1 sex partner, who answered that they did not know, or for whom data were missing.

§The reference group consisted of the subjects who did not have a new sex partner, who answered that they did not know, or for whom data were missing.

¶The reference group consisted of the subjects who had a partner who always used condoms.

recruits would be tested if they were 25 years of age or less or if they reported on a questionnaire having had more than one sex partner or a new sex partner in the previous 90 days, having had a partner who did not use condoms in the previous 90 days, or having a history of sexually transmitted disease. Screening according to these criteria would still require testing 100 percent of the population. If a questionnaire could be avoided and young age (25 years or less) alone was the screening criterion, 87.9 percent (11,603 of 13,204) of the population would need to be tested and 95.3 percent (1162 of 1219) of the positive subjects would be identified.

Geographic Variation in Prevalence

There was considerable variation in the prevalence of chlamydial infection according to the state of origin of the recruits ($F < 0.001$ by one-way analysis of variance). The prevalence was more than 15 percent for recruits from South Carolina, Georgia, Alabama, Louisiana, and Mississippi. For New Jersey, North Carolina, Kentucky, Texas, Oklahoma, and Arkansas, the prevalence was 10 to 15 percent, and for 17 other states and Puerto Rico, it was 5 to 10 percent. For five states (Washington, Oregon, Minnesota, Arizona, and Massachusetts), the prevalence was less than 5 percent. Fewer than 100 recruits were tested from each of 17 states, 3 territories, and the District of Columbia, and prevalence figures from these areas were therefore not included in the analysis. The prevalence for the five states with the highest prevalence and the five states with the lowest prevalence differed significantly, since the 95 percent confidence intervals for prevalence did not overlap.

DISCUSSION

Although the diagnosis and treatment of sexually transmitted diseases has always presented a challenge, there has been no routine screening of recruits for chlamydial infections at entry into the U.S. Army.²¹ Because most chlamydial infections are asymptomatic in women and because the sequelae of disease present a severe and costly burden, screening women at entry into the Army is an appropriate way to identify infections early and to explore opportunities for a control program.^{1,6,7,22}

Civilian chlamydia-control programs have sought to identify criteria for selective screening.^{23–27} Most of these control programs have used diagnostic assays that require pelvic examinations and cervical specimens.²⁵ However, it has recently been shown that testing urine specimens by DNA-amplification techniques is cost effective for screening large numbers of persons in different settings.^{15,28} We used this new technique to determine the prevalence of chlamydia and to identify screening criteria for a program to control chlamydia in the military.^{10,12,14} Collection of urine specimens in this study was highly acceptable and easily implemented.

Using the ligase chain reaction with urine samples, we found a high prevalence of *C. trachomatis* infection (9.2 percent). This prevalence was higher than that observed in family-planning clinics²⁸ but not as high as that reported in some adolescent health clinics.^{29,30} Our data agree with those from previous studies of chlamydial infections in Army women, in which prevalence rates ranged from 8.2 percent to 9.8 percent.^{17,18} In one large, community-based screening study, the overall prevalence of chlamydia in young women was 8.6 percent, as detected by the urine ligase chain reaction, a prevalence similar to that found in our study.³¹ Because our population was not clinic-based and was not made up of women seeking health care, the finding of such a high prevalence in these women warrants the institution of a control program for the routine identification and treatment of chlamydial infections in order to prevent sequelae and transmission to sex partners.⁸

The study population consisted of a young, sexually active group of female recruits with sexual risk factors known to be associated with chlamydial infection.²⁵ Although 9.1 percent of the subjects reported having had chlamydial infection in the past, this factor was not associated with the risk of current infection. The highest prevalence was observed among 17-year-olds. This prevalence is similar to comparable age-specific rates in other studies, confirming that young age is associated with chlamydial infection.^{28,31} In our study, young age was associated with being chlamydia-positive in both univariate and multivariate analyses (odds ratio, 3.0). In order to include more positive subjects, we used an age cutoff of 25 years, which allowed the detection of 95.3 per-

cent of the chlamydial infections. Other studies have supported age-based screening for chlamydia.^{27,31,32}

Thus, for this group of female recruits coming from a civilian background, who were tested within three days of starting basic training, young age alone can be recommended as a single indicator of who should be tested for chlamydial infection. Other models considered in this study offered high sensitivity, but the models were more complex and required valid sexual-risk histories. We documented 13 chlamydial infections (prevalence, 1.4 percent) among 914 recruits who denied being sexually active, as well as chlamydial infections in 8.4 percent of those who reported that their partners consistently used condoms. These figures indicate that self-reported sexual-risk histories are not always valid.³³ The lower prevalence of chlamydial infection among recruits for whom the data on condom use were missing, or who indicated on their questionnaires that they did not know whether their partners always used condoms, may be due to lack of sexual activity, because 58.6 percent of the 684 recruits in these categories reported that they had never had vaginal sex. There is a fixed laboratory budget available for population screening in the Army. Young age is the simplest, least expensive, and most easily documented risk factor on which to base a recommendation for a screening program, as well as being highly sensitive. Alternatively, since the use of age as a selective screening criterion would have missed 4.7 percent of the infections, universal screening might be more cost effective from a societal perspective, and future studies of cost effectiveness are warranted.²⁸

This was one of the largest programs for screening young, sexually active subjects that was not clinic-based and whose results were derived from urine DNA-amplification assays. The geographic variation in prevalence was striking. From more than 15 percent in the five states with the highest prevalence to less than 5 percent in the five states with the lowest, these differences may reflect the levels of disease burden in certain states. These regional variations also appear to reflect regional differences in chlamydial disease, as reported by the Centers for Disease Control and Prevention.^{34,35} For example, the prevalence in North Carolina reportedly varied from 10 percent to 17 percent.³⁵ The prevalence is lower in regions such as Wisconsin and Washington State, where clinic-based chlamydia-control programs are in place and where declining rates of prevalence of chlamydia have been reported.^{26,27,32,36} In our study, the prevalence was 11.3 percent for North Carolina and 3.8 percent for Washington State. Our data imply that chlamydial infection remains common in young women across the United States. With a volunteer rate of 80 percent among women who were approached and representation from 50 states and 4 territories, our study had a wide geographic sampling.

One limitation of our study is that it is not known whether the prevalence of risk factors for chlamydial infection differs between young women who decide to join the military and those who do not. However, the demographic and sexual risk-factor characteristics of our subjects appear to be similar to those of other regional and clinic-based populations,²⁵ as well as those from a large, community-based study.³¹ An additional limitation is that the nonvolunteers in our study differed from the volunteers with regard to sexual risk factors for chlamydia. However, the nonvolunteers represented a group who were mostly sexually active, who had had new sex partners in the previous 90 days, and whose partners did not use condoms. Thus, their risk of chlamydial infection may have been as high as that of the subjects in our study.

Although amplified-DNA tests are more expensive than traditional nonculture tests, the savings associated with not having to have a clinician collect specimens from a pelvic examination and the advantages of being able to use urine as a diagnostic specimen may outweigh the extra cost of the test.¹⁵ In addition, it has been demonstrated that amplified-DNA testing of urine specimens is cost effective, and treating chlamydial infections prevents serious complications such as pelvic inflammatory disease, ectopic pregnancy, and infertility.^{15,28,37}

In conclusion, our study indicates that with the limited funding available at present, young age (25 years or less) would be the best criterion on which to base a screening program using amplified-DNA testing of urine for female Army recruits and perhaps for other young women. Institution of such a control program has the potential to reduce drastically the burden of chlamydial disease in the U.S. Army and to prevent morbidity due to these infections.³⁷

Supported by a grant (DAMD17-95-1-5064) from the Department of the Army.

The opinions expressed in this article are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense.

We are indebted to Dr. Daniel Scharfstein, Mary Sheppard, Dr. Guillermo Madico, and Dr. Anne Rompalo for statistical assistance; to Kathryn Kacena for data entry; to Diane Perkins, Sandra Leister, Dien Pham, and Graciela Jaschek for performance of the laboratory tests; to Lt. Col. Cynthia Dodd, Lt. Col. (ret.) Delois Daniels, and Kimberly Riley for treatment of infected women and processing of specimens; and to Patricia Buist for assistance in the preparation of the manuscript.

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